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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATH

WE record with regret the death of

James Rawson Walmsley.

NORTH OF ENGLAND SECTION

THE twenty-first Summer Meeting of the Section was held at the Old Swan Hotel, Harrogate, from Friday, June 6th, to Monday, June 9th, 1958.

The Chairman of the Section, Mr. A. N. Leather, B.Sc., F.R.I.C., presided over an Ordinary Meeting at 10.15 a.m. on Saturday, June 7th, at which Mr. R. J. Gardner, Publicity Officer of Imperial Chemical Industries Ltd. (Fibres Division) spoke on "Terylene," illustrating the formation of synthetic fibres and the discovery and uses of Terylene by means of a colour film and model display.

On the Saturday evening the party saw "The House by the Lake," at the Grand Theatre, Leeds, and made a coach tour to Wharfedale, Burnsall and Bolton Abbey on the Sunday afternoon.

MICROCHEMISTRY GROUP

THE fifteenth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, June 18th, 1958, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by Dr. G. F. Hodsman, B.Sc., A.Inst.P.

A discussion on "The Microdetermination of Elements other than C,H,O,N,S and Cl,Br,I in Organic Compounds" was opened by Alison M. G. Macdonald, B.Sc., M.Sc., Ph.D., A.R.I.C., and R. Belcher, D.Sc., Ph.D., F.R.I.C., F.Inst.F.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held on Thursday, May 22nd, 1958, when members visited the new Pharmaceutical Research Laboratories of Imperial Chemical Industries Ltd., at Alderley Park, Cheshire.

A description of the Research Department by the Division's External Relations Officer preceded a tour of the laboratories during which an opportunity was given to see and discuss the work of the various sections visited.

At the close of the meeting, thanks on behalf of the Group for a most instructive and enjoyable visit were expressed by the Vice-Chairman, Dr. J. I. M. Jones, D.Sc., F.R.I.C.

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Polynuclear Hydrocarbons in Tobacco and Tobacco Smoke

Part I. 3:4-Benzopyrene

By H. R. BENTLEY and J. G. BURGAN

(*The Imperial Tobacco Company Ltd., Research Department, Raleigh Road, Bristol*, 3)

A method is described for determining the concentration of 3:4-benzopyrene in tobacco and in tobacco-smoke condensate by using the fluorescence spectrum of the hydrocarbon.

It has been suspected for many years, largely by analogy with coal tar, that the water-insoluble "tar" forming the condensable fraction of tobacco smoke would be found to contain polynuclear aromatic hydrocarbons.

An essential difference, however, between normal tobacco smoke and coal tar is that, whereas the latter is the by-product of a carbonisation process occurring in the absence of air, tobacco smoke contains the products of combustion of leaf constituents and of distillation of volatile substances in the presence of air. Considerable amounts of leaf constituents are also carried over into the smoke by a process of steam-distillation and entrainment, and, since these substances are removed rapidly from the burning zone, they are to a large extent chemically unaltered.

Polynuclear aromatic hydrocarbons are thought to be formed during pyrolysis of many substances in a restricted supply of air between about 750° and 1600° C by a process involving first a breakdown into methylene radicles and hydrogen.[1] The methylene radicles dimerise to ethylene, which breaks down further to hydrogen and "nascent acetylene." This partly decomposes to carbon, hydrogen and methane and partly polymerises to polynuclear aromatic hydrocarbons with further loss of hydrogen. A mechanism of this sort may explain the formation of carcinogenic tars during the carbonisation in the absence of air of the organic materials that were tested by Kennaway[2,3] and Kennaway and Sampson.[4] These carbonisation conditions are, however, not representative of those in the burning zone of a cigarette, and normal tobacco smoke is, *a priori*, unlikely to contain appreciable amounts of polynuclear aromatic hydrocarbons. This may not be so in tars produced by heating tobacco in closed stills,[5,6,7,8,9] which may be expected to resemble carbonisation tars more closely, and this essential difference was noted by some of the earlier workers[10,11] and also in more recent times.[12]

Because of its important biological effects, and also because of the comparative ease with which it can be recognised in mixtures, attention has been directed first to 3:4-benzopyrene. However, many workers who analysed tobacco tar by methods applicable to coal tar failed to detect the hydrocarbon.[11,13,14,15] An early report by Roffo[6] that tobacco tar contained 3:4-benzopyrene arose by a confusion of nomenclature. The hydrocarbon for which spectroscopic evidence was obtained by Roffo was, in fact, 1:2-benzopyrene, which he believed to be the highly carcinogenic isomer.

It is now evident that one of the reasons for the earlier failures to detect 3:4-benzopyrene in tobacco tar was that the order of magnitude of the concentration present was not appreciated. It has recently become clear that the smoke from cigarettes smoked under conditions simulating the human habit does in fact contain small amounts of 3:4-benzopyrene. There is, however, little agreement among workers in this field as to the amounts present. For example, Cooper and Lindsey[16] give a figure of about 1 μg per 100 cigarettes smoked, equivalent to 0·2 p.p.m. by weight of condensable material; Wynder[17] gives the concentration as 2 p.p.m. by weight of condensable matter; Alvord and Cardon[18] found a range of 8 to 18 μg per 100 cigarettes smoked, equivalent to 1·6 to 3·6 p.p.m. by weight of condensable matter; Latarjet, Cuzin, Hubert-Habart, Muel and Royer[19] found 1·2 μg per 100 cigarettes smoked, equivalent to 0·2 p.p.m. by weight of condensable matter; and Bonnet and Neukomm[20] found 2·2 μg per 100 cigarettes smoked, equivalent to about 0·4 p.p.m. by weight of condensable matter.

Differences in smoking technique are probably responsible for some of these variations. In the work now reported, cigarettes have been smoked under conditions that are thought to resemble most closely those of the human habit.[21]

In our experience, which we believe to be general, it has not yet been possible to isolate pure polynuclear hydrocarbons from cigarette-smoke condensate. Even after extensive chromatographic separation, the 3:4-benzopyrene-containing fractions contain a large excess of extraneous material, which contributes an intense background absorption in the 300 to 400-mμ region and an intense fluorescence at a somewhat longer wavelength. The typical appearance of the absorption spectrum of a purified 3:4-benzopyrene-containing fraction is shown in Fig. 1. In this, the absorption peaks at 365 and 385 mμ, which are characteristic of the hydrocarbon, can be seen as small inflexions against a generalised background absorption. A method for determining substances by the heights of absorption peaks under these conditions is that of Morton and Stubbs,[22] which has been applied to the determination of vitamin A in fish oils and of anthracene in petroleum. The small and indeterminate peaks obtained from purified cigarette-smoke fractions, however, make it impossible to use this method for 3:4-benzopyrene. For example, a requirement of the method of Morton and Stubbs is that linear irrelevant absorption must be assumed to be present over the region of the peak used in the determination. This assumption cannot be made for certain fractions of tobacco tar. As will be shown later, the analytical method finally adopted depends on the use of fractions that show the characteristic banded fluorescence spectrum of 3:4-benzopyrene, and these fractions invariably also show small inflexions in the absorption spectrum characteristic of 3:4-benzopyrene. On the other hand, fractions closely adjacent to these on the chromatogram have been found to show apparently typical 3:4-benzopyrene inflexions without displaying the characteristic fluorescence bands of the hydrocarbon. The possibility that the absence of typical fluorescence in these fractions might be due to quenching was excluded by the addition of small amounts of pure 3:4-benzopyrene; the characteristic bands then appeared with the correct intensity. It cannot, therefore, be assumed that the background absorption is linear for the purpose of measuring peak heights.

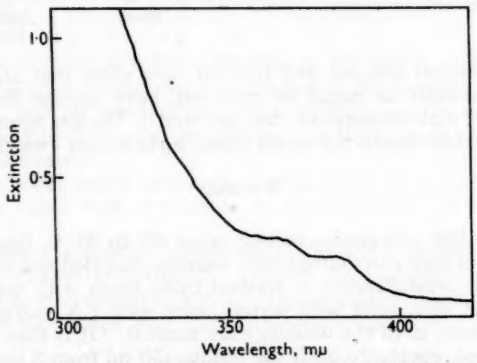


Fig. 1. Absorption spectrum of purified 3:4-benzopyrene-containing fraction

The values reported hitherto in the literature for the 3:4-benzopyrene content of tobacco smoke have all been found by the absorption method. Because they show considerable discrepancies, it was thought that it would be useful to develop, for comparison, an analytical method based on an entirely different principle. The proposed method, based on fluorescence, has therefore been devised. The application of the method to some problems of current interest is shown by the results in Table I.

For mixed cigarettes representative of those that have a large sale in the United Kingdom, a large number of replicate determinations permits the reproducibility of the method to be assessed. For the twenty-five results listed in the first section of Table I, the mean maximum 3:4-benzopyrene content per 500 g of cigarettes is 4·9 μg, with a range 1·5 to 8·0 μg. This concentration is equivalent to about 0·2 p.p.m. by weight of condensable matter and agrees with the results of Cooper and Lindsey,[16] Waller[15] and Latarjet, Cuzin, Hubert-Habart, Muel and Royer.[19] The concentrations found by other workers, which are considerably higher than this, are therefore not typical of the cigarettes on sale in the United Kingdom smoked under our conditions.

The results listed in Table I also show that there is no difference in the 3:4-benzopyrene content of the smoke from cigarettes containing only American or Rhodesian tobacco, although the chemical composition of these two types of leaf differs appreciably. Also, there is no more 3:4-benzopyrene in the smoke from cigarettes made entirely of cut tobacco stems, which have a very high cellulose and lignin content and are practically free from alkaloids.

TABLE I

DETERMINATION OF 3:4-BENZOPYRENE IN TOBACCO SMOKE, LEAF AND STEM

Source	Material analysed	Maximum 3:4-benzopyrene content per 500 g of source, μg		
Mixed cigarettes typical of current United Kingdom production	Smoke	6·0	3·0	8·0
		1·5	5·0	2·0
		4·0	1·5	3·0
		6·0	8·0	7·0
		5·5	3·0	4·0
		2·7	7·0	3·0
		5·0	3·0	6·0
		7·0	7·0	8·0
				7·0
	Tobacco ..	2·0	2·75	5·0
American cigarettes	Smoke ..	5·0	2·5	3·0
		2·0		
Rhodesian cigarettes	Smoke ..	3·0	3·5	6·0
		2·5		
	Tobacco ..	2·0	3·0	2·5
Cigarettes made from tobacco stems	Smoke ..	5·0	4·0	4·0
		5·0		
	Stem ..	Not found	0·2	

The figures for unburnt tobacco and tobacco stem show that after normal curing and manufacture these materials, as might be expected, have become contaminated with 3:4-benzopyrene by contact with atmospheric dust and soot. The low figure for stem as compared with lamina is presumably due to the much lower surface area - weight ratio of the former.

METHOD

REAGENTS—

Light petroleum—Light petroleum, boiling range 40° to 60° C, free from aromatic hydrocarbons, is percolated through chromatographic alumina, distilled and stored over sodium wire.

Benzene—Benzene (crystallisable) is washed three times with concentrated analytical-reagent grade sulphuric acid, once with water, twice with 2 *N* sodium hydroxide solution and then again with water until the washings are neutral. It is then dried over anhydrous sodium sulphate, distilled repeatedly until the residue (50 ml from 2 litres) does not fluoresce and stored over sodium wire.

Diethyl ether—Anaesthetic ether B.P. is dried over sodium wire.

Acetone—Laboratory-reagent grade acetone is used without further purification.

Alumina—Woelm alumina, neutral grade, activity 1, is used.

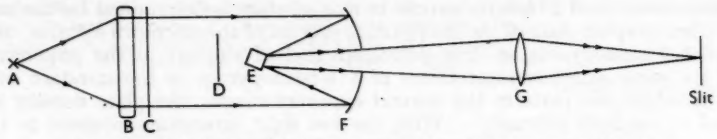
3:4-*Benzopyrene solution*—A solution of the commercial material in light petroleum is percolated through alumina (Woelm neutral grade), and the principal fluorescent band is eluted with 40 per cent. v/v of benzene in light petroleum. The hydrocarbon is recovered from the eluate by evaporation and then recrystallised from light petroleum and dried *in vacuo*. The product has $\log(\epsilon_{382m\mu}) = 4{\cdot}46$ (literature 4·47).

PROCEDURE FOR RECORDING FLUORESCENCE SPECTRA—

Fluorescence spectra are obtained with a Hilger medium-quartz spectrograph and photographed. In the examination of chromatographic fractions for the presence of 3:4-benzopyrene, Ilford HPS plates (5 inches × 4 inches) are used with an exposure time of 1 to 4 minutes. In the determination of 3:4-benzopyrene, Ilford HP3 plates (5 inches × 4 inches) are used with an exposure time of 2 to 12 minutes. The optical system used is shown in Fig. 2.

The source of exciting radiation, A, is a "black-glass" 125-watt mercury-arc lamp used in conjunction with a polished Wood's glass filter, C. This transmits a group of lines in the 365-mμ region, which excites the visible fluorescence of many polycyclic hydrocarbons.

By means of a large glass condensing lens, B, and a concave mirror, F, arranged as shown, the exciting radiation is focused on the sample cell, E. This is a 1-cm quartz cell, with lid, turned slightly off axis so as to deflect as much stray mercury light as possible from the spectrograph slit. To reduce the amount of stray mercury light still further, a matt-black shield, D, is placed in front of the cell.



A = 125-watt "black-glass" mercury-arc lamp (G.E.C. type MBW/U)
B = Condensing lens
C = Wood's glass filter (Chance OX1, 2 mm thick)
D = Matt-black shield
E = Quartz sample cell
F = Concave mirror
G = Lens of short focal length

Fig. 2. Optical system used for recording fluorescence spectra

The fluorescent light emitted from the cell passes through an aperture in the centre of the concave mirror and is focused on the spectrograph slit by means of a lens of short focal length, G. The whole of the optical system is conveniently mounted on the optical bench of a spectrograph.

PROCEDURE FOR DETERMINING 3:4-BENZOPYRENE IN CIGARETTE SMOKE—

The cigarettes used in this work are conditioned at 60 per cent. relative humidity and 70° F before smoking, and are smoked under the standard conditions described elsewhere.[21] The smoke is collected by electrostatic precipitation, use being made of the automatic smoking machine described elsewhere.[21]

The precipitated smoke solids (about 25 g) from 500 g of cigarettes are extracted from the autosmoker glass tubes with a mixture of equal volumes of diethyl ether and 2 *N* hydrochloric acid.

The combined ether extracts are separated and washed, successively, with four 100-ml portions of 2 *N* hydrochloric acid, 100 ml of water, four 100-ml portions of 2 *N* sodium hydroxide and three 100-ml portions of water. The solution of the neutral fraction of smoke condensate in ether is then dried over anhydrous sodium sulphate, filtered, and evaporated on a steam-bath.

The residue is recovered by evaporation three times from successive small volumes of light petroleum; it is then dissolved in 50 ml of light petroleum and transferred to a column of 115 g of alumina in a glass tube of 28 mm diameter, protected from direct sunlight.

The chromatogram is developed with 500 ml of light petroleum and then, successively, with 250-ml portions of 10, 20 and 30 per cent. v/v benzene - light petroleum mixtures and finally with sufficient 100-ml portions of 40 per cent. v/v benzene - light petroleum mixture to elute all the 3:4-benzopyrene from the column.

Each fraction is evaporated to dryness, care being taken to remove all traces of benzene, and re-dissolved in 5 ml of light petroleum. The fluorescence spectrum of each fraction is recorded, and those fractions containing 3:4-benzopyrene are combined.

The combined fractions are concentrated to small volume and transferred to a column of 10 g of alumina in a glass tube of 16 mm diameter, protected from direct sunlight. The chromatogram is developed with 50 ml of light petroleum, successive 25-ml portions of 10, 20 and 30 per cent. v/v benzene - light petroleum mixtures and finally sufficient 25-ml portions of 40 per cent. v/v benzene - light petroleum mixture to elute all the 3:4-benzopyrene. The fractions are evaporated to dryness, care again being taken to remove all benzene, re-dissolved in 5 ml of light petroleum and examined for the presence of 3:4-benzopyrene as before. Fractions containing 3:4-benzopyrene are combined and evaporated on a steam-bath.

The residue is dissolved in a suitable known volume of light petroleum. This volume depends on the amount of 3:4-benzopyrene present, but the concentration of 3:4-benzopyrene in it must be such that the following requirements are met—

(*i*) The bands in the fluorescence spectrum at 403, 408 and 427 mμ are visible.

(*ii*) The fluorescence due to the maximum amounts of 3:4-benzopyrene to be subsequently added to aliquots for the purpose of determination must not be appreciably quenched in the solution. When necessary, this requirement is checked by means of a microphotometer, use being made of the strong 403-mμ band.

The concentration of 3:4-benzopyrene in this solution is determined by the comparison on a single photographic plate of the fluorescence spectra of the unknown solution, of standard solutions of 3:4-benzopyrene in light petroleum and of aliquots of the unknown solution containing the same added concentrations of 3:4-benzopyrene as the standard solutions.

For a photographic plate in the normal exposure ranges, the plate density is directly proportional to log(light intensity). With the low light intensities involved in this work, however, the plates are very much underexposed and the increase in plate density with increasing light intensity, and hence with increasing concentration of 3:4-benzopyrene, is found to be nearly linear for solutions of the pure hydrocarbon in light petroleum. Hence, by visual comparison of the spectra of the unknown solution and the solutions containing added 3:4-benzopyrene, it is possible to determine the level of added 3:4-benzopyrene at which the concentration of the hydrocarbon in the unknown solution has been doubled, and hence what this concentration is.

With the proposed procedure, the final recovery of pure 3:4-benzopyrene added to solutions of smoke condensate in diethyl ether before the initial extraction with hydrochloric acid is 85 to 90 per cent., which provides a correction factor for determinations on normal smoke condensates.

PROCEDURE FOR DETERMINING 3:4-BENZOPYRENE IN LEAF AND CIGARETTE TOBACCO—

The leaf is stemmed and cut before extraction. Manufactured cigarette tobacco is extracted without further preparation.

Cut leaf or cigarette tobacco (200 g) is extracted with acetone for 4 hours in a Soxhlet extractor. The extract is evaporated under reduced pressure on a steam-bath, and the residue is hydrolysed by boiling under reflux for 2 hours with 100 ml of 10 per cent. w/v ethanolic potassium hydroxide. The resulting solution is concentrated under reduced pressure on a steam-bath, diluted with water and repeatedly extracted with diethyl ether.

The combined ether extracts are washed, successively, with three 50-ml portions of 2 *N* hydrochloric acid, 50 ml of water, three 50-ml portions of 2 *N* sodium hydroxide and three 50-ml portions of water; they are then dried with anhydrous sodium sulphate and evaporated under reduced pressure on a steam-bath. The residue is recovered by evaporation three times from successive small volumes of light petroleum, dissolved in 50 ml of light petroleum and transferred to a column of 115 g of alumina, exactly as for the analysis of smoke condensate. With leaf extracts it is usually unnecessary to re-chromatograph the combined 3:4-benzopyrene-containing fractions. The 3:4-benzopyrene content of the combined fractions can then frequently be determined by direct comparison on a photographic plate of the fluorescence spectrum of the unknown solution with the spectra of standard solutions of the hydrocarbon; otherwise the determination is carried out by the method described for smoke condensate. For pure 3:4-benzopyrene added to tobacco before extraction and hydrolysis of the extract, the recovery from the final chromatogram fractions is 75 per cent., which provides a correction factor for determinations on normal tobaccos.

We thank the Directors of The Imperial Tobacco Company (of Great Britain and Ireland) Ltd. for permission to publish this paper.

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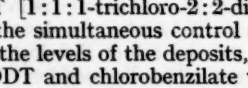
Determination of DDT and Chlorobenzilate Occurring Together in Spray Deposits

By E. A. BAKER and E. JOHN SKERRETT
(*Research Station, Long Ashton, Bristol*)

Mixtures of DDT and chlorobenzilate from spray deposits on plant surfaces are separated on alumina columns, and the two constituents are determined by procedures based on the colours developed from their nitration products.

Chlorobenzilate (ethyl 4:4′-dichlorobenzilate, **I**) is a non-phytocidal spray material developed for the control of mite infestations of plants. It has been used successfully for controlling the citrus-bud mite and may prove to be of value against other species, such as the big-bud mite of blackcurrants. For this latter purpose it would be convenient to use DDT [1:1:1-trichloro-2:2-di(*p*-chlorophenyl)ethane, **II**] in conjunction with the acaricide for the simultaneous control of the currant-leaf midge. In order to assess the distribution and the levels of the deposits, it is necessary to have available methods for the determination of DDT and chlorobenzilate when used together in such sprays.

Several methods have been described for the determination of DDT[1,2,3] and chlorobenzilate[4,5] occurring singly in spray deposits on plants, but Harris[4] has been the only worker to consider mixtures of the two. He separated chlorobenzilate from DDT by treatment with ethanolic potassium hydroxide solution. This reagent hydrolysed the former to the potassium salt of the acid **III** and dehydrohalogenated the DDT to DDX (**IV**).



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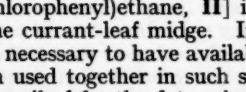

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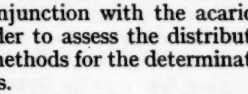

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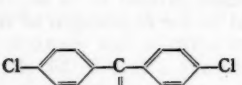

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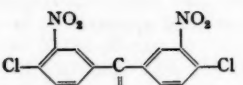

(V)

After dilution with water, the DDX was removed with light petroleum, and, after acidification, the acid **III** was extracted with diethyl ether. The acid was then nitrated to

a tetranitro compound (**V**), which was determined colorimetrically. The method did not measure the DDT present, but our preliminary experiments showed that the DDX removed in the light petroleum washes could have been used to determine the original level of DDT. However, chromatographic methods of separation were investigated in order to reduce both time and apparatus requirements. It was thought that such a separation was feasible, as chlorobenzilate, with its tertiary carbinol hydroxyl group, might be strongly adsorbed on a hydrophilic column through which DDT would pass.

Experimental

Initial experiments with silica and alumina columns indicated a quantitative separation of chlorobenzilate from DDT in carbon tetrachloride solution, the final traces of DDT being eluted from the column with small portions of carbon tetrachloride. Unfortunately, in later experiments with plant extracts, silica columns failed to retain materials that interfered with the colorimetric determination of DDT, and the use of this chromatographic medium had to be abandoned.

Incomplete recoveries of chlorobenzilate from alumina columns in early stages of the work were attributed to the formation of the insoluble sodium salt of the acid, **III**, with traces of alkali in the alumina. This trouble was overcome by carefully neutralising, washing, drying and conditioning the alumina before use.[6]

During the preliminary experiments, it was found to be desirable to modify slightly the established methods for the individual toxicants. In Harris's method[4] for the determination of chlorobenzilate, the nitration mixture is slowly warmed over a period of 30 minutes to 85° C and then heated in a steam-bath for 1 hour. It was found that the initial heating period could be omitted and the flask containing the reaction mixture placed directly into boiling water. However, under these conditions a minimum heating period of 1 hour was necessary. Saturated sodium sulphate solution was used to dilute the reaction mixture; this gave sharper and much more rapid separations during the ether extractions. Difficulties were caused by the growth of *Fusarium* spp. in the sodium sulphate solution, which was prevented by bubbling sulphur dioxide through the solution for a short time. This development of mould was rather surprising, although a few analogous instances[7,8] have been reported. 2-Ethoxyethanol (ethyl Cellosolve) was preferred to benezene as solvent for the nitration product, which has been shown[9] to be a tetranitrobenzophenone derivative (**V**). Methanolic potassium hydroxide solution was preferred for the colour development, as it gave less trouble from carbonate precipitation than did the ethanolic solution. The maximum colour developed after 30 minutes and was stable for a further 2 hours.

Determination of DDT was based on the nitration method of Schechter, Soloway, Hayes and Haller.[1] When solutions of plant extract were evaporated, it was found to be desirable to add a little oxalic acid to prevent loss of insecticide. (The stearic acid used by Harris for a similar purpose in the determination of chlorobenzilate was found to cause interference in the final colour development.) The time of nitration was reduced to 10 minutes,[10] and the cooled acid mixture was diluted with saturated sodium sulphate solution. 2-Ethoxyethanol was again used to dissolve the nitration product, the final blue colour being developed with 5 per cent. ethanolic potassium hydroxide solution and measured at 390 mμ with a Unicam SP600 spectrophotometer.

In both procedures it was found that traces of rubber, silicone and Apiezon stopcock lubricants interfered with the final dissolution of the nitro compounds. However, a stiff paste of bentonite and analytical-reagent grade glycerol proved to be satisfactory, and the addition of a glass bead appeared to be a further aid to the dissolution of residues.

Method

Apparatus—

All-glass apparatus is used throughout.

Chromatographic columns are prepared in tubes, as shown in Fig. 1. The small glass projections are of use when the tubes are positioned in the Bolton extractors during the extraction stage.

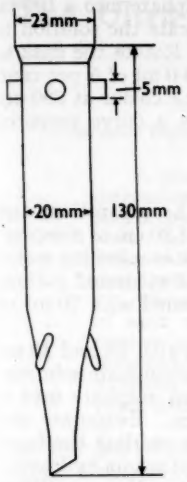

Fig. 1. Modified chromatographic tube

REAGENTS—

Use a Widmer column with a proportionating head for all fractionations.

Diethyl ether—Purify technical grade ether by fractionation over sodium.

Methanol—Purify B.P. grade methanol by fractionation after the addition of sodium.

Ethanol—Purify technical grade absolute ethanol by fractionation after the addition of sodium.

2-*Ethoxyethanol*—Purify technical grade ethyl Cellosolve by distillation after the addition of sodium.

Carbon tetrachloride—Purify the analytical-reagent grade material by fractionation.

Nitration mixture—Cautiously add an equal volume of analytical-reagent grade concentrated sulphuric acid to well stirred fuming nitric acid, also of analytical-reagent grade.

Stearic acid solution—Twice recrystallise stearic acid from ethanol, and prepare a 0·5 per cent. solution in light petroleum (boiling range 40° to 60° C).

Oxalic acid solution—Prepare a 0·5 per cent. solution of analytical-reagent grade oxalic acid in acetone.

Sodium sulphate solution, saturated—Saturate distilled water with sodium sulphate, and pass sulphur dioxide through the solution for a few minutes.

Methanolic potassium hydroxide solution, 5 per cent.—Freshly prepare this solution in the cold, and filter before use.

Ethanolic potassium hydroxide solution, 5 per cent.—Freshly prepare this solution in the cold, and filter before use.

Alumina—Stir 500 g of H-grade alumina with 300 ml of 0·2 per cent. v/v hydrochloric acid for 2 hours. Allow the mixture to settle, and then decant the supernatant liquid. Repeat this procedure with a further 300 ml of 0·2 per cent. hydrochloric acid. Wash the alumina twice with 300-ml portions of distilled water, stir it with 300 ml of distilled water and then with 300 ml of 0·3 per cent. v/v acetic acid for 1 hour. Filter the alumina on a Buchner funnel, and dry at 120° C. Break up any lumps, spread in a thin layer on a silica tray and heat at 600° C for 3 hours. Allow to cool from approximately 200° C in a closed container, and condition the alumina to Brockmann activity III[6] by placing a beaker containing 15 ml of water in the container for 3 days.

Stopcock lubricant—Add sufficient bentonite to analytical-reagent grade glycerol to form a viscous paste after being stirred.

PROCEDURE FOR SEPARATING THE MIXTURE—

Extract 1 g of the plant material for 15 minutes in a Soxhlet extractor with 25 ml of carbon tetrachloride. Lightly plug the bottom of a chromatographic tube with cotton-wool that has been extracted with hot ethanol, add 5 g of alumina and tap the column to ensure that the packing is uniform. Use another small plug of cotton-wool to prevent displacement of the top of the column. Pour the cooled plant extract on to the column, and wash with five 5-ml portions of carbon tetrachloride. Collect the eluate, and use it for the determination of DDT. Place the column in a Bolton extractor, and extract for 2 hours with 35 ml of hot ethanol. Use this extract for the determination of chlorobenzilate.

PROCEDURE FOR DETERMINING DDT—

Add 2 ml of oxalic acid solution and a glass bead to the eluate, and evaporate to dryness at approximately 55° C and a pressure of 20 cm of mercury. To the cooled residue add 2 ml of nitration mixture, and carefully rotate the flask to wet any particles of solid. Immerse the flask in a boiling-water bath for 10 minutes, occasionally swirling the contents. Cool the flask in an ice - water mixture, and add 75 ml of saturated sodium sulphate solution. Transfer the contents of the flask to a separating funnel with 70 ml of diethyl ether. Shake, discard the aqueous layer, and wash the ethereal layer successively with 25 and 10 ml of 5 per cent. potassium hydroxide solution and 15 ml of saturated sodium sulphate solution.

Run the ethereal solution through a 15-mm layer of anhydrous sodium sulphate into a 100-ml round-bottomed flask containing a glass bead of diameter 5 mm. Evaporate the solution to dryness in a water bath at 55° C, and add 0·5 ml of 2-ethoxyethanol. Rotate the flask so that the bead assists the dissolution of the residue, cool, and then add 10·0 ml of 5 per cent. ethanolic potassium hydroxide solution. After 5 minutes, measure the colour at 390 mμ with a Unicam SP600 spectrophotometer, and interpolate the result on a curve prepared from standard mixtures of plant extracts and DDT (m.p. 107·5° C).

PROCEDURE FOR DETERMINING CHLOROBENZILATE—

Add 2 ml of stearic acid solution to the extract obtained from the separation, and evaporate the mixture to dryness at approximately 55° C and a pressure of 20 cm of mercury. To the cooled residue add 5 ml of nitration mixture, and immerse the flask in a boiling-water bath for 1 hour. Cool the flask in an ice - water mixture, and add 75 ml of saturated sodium sulphate solution. Transfer the contents of the flask to a separating funnel with 70 ml of diethyl ether, and shake.

Discard the aqueous layer, and wash the ethereal layer successively with 25 and 10 ml of 5 per cent. potassium hydroxide solution and 15 ml of saturated sodium sulphate solution. Run the ethereal solution through a 15-mm layer of anhydrous sodium sulphate into a 100-ml round-bottomed flask containing a glass bead of diameter 5 mm. Evaporate the solution to dryness at 55° C, and add 0·5 ml of 2-ethoxyethanol. After swirling the bead round the flask to assist dissolution, add 10·0 ml of 5 per cent. methanolic potassium hydroxide solution. After 30 minutes, measure the colour developed at 538 mμ with a Unicam SP600 spectrophotometer, and determine the chlorobenzilate by using a curve prepared from standard mixtures of plant extracts and chlorobenzilate (m.p. 37° to 38·5° C).

RESULTS

Mixtures prepared by adding known amounts of DDT and chlorobenzilate to extracts of 1 g of untreated leaves were analysed by the proposed method. The results shown in Table I indicate good recoveries.

TABLE I

RECOVERIES OF DDT AND CHLOROBENZILATE FROM SYNTHETIC MIXTURES

Amount of DDT added, μg	Amount of chlorobenzilate added, μg	Amount of DDT found, μg	Amount of chlorobenzilate found, μg
10	10	10·5, 10·0	10·0, 9·8
20	20	21·0, 20·5	19·9, 19·7
50	50	49·7, 49·5	49·7, 49·5
75	75	73·5, 72·5	72·5, 72·0
100	100	98·0, 97·0	96·0, 95·0
200	200	193, 192	197, 196

We thank Professor H. G. H. Kearns and Dr. J. T. Martin for their interest in the work, and Messrs. Geigy Ltd. for the gift of a sample of chlorobenzilate.

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The Ultra-violet Spectrophotometric Determination of Sugars and Uronic Acids

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A simple ultra-violet spectrophotometric method is described for the determination of microgram amounts of aldo- and keto-hexoses, pentoses and uronic acids in a single pure solution. The sugars and uronic acids are heated in 90 per cent. sulphuric acid solution, and the optical densities are measured at the appropriate wavelength. The characteristics of the absorption spectra of the reaction products are given.

The method is particularly suited to the determination by one procedure of each of a series of carbohydrates that has been separated by paper chromatography. Interference by the usual chromatographic solvents, with the exception of *n*-butyl alcohol, is negligible. Experiments in which known mixtures of sugars were separated on paper chromatograms, eluted from the paper and analysed by the method show satisfactory recoveries.

THE reaction of sulphuric acid with carbohydrates has been extensively studied in recent years. Holzman, MacAllister and Niemann[1] observed the spectral characteristics of some monosaccharides in 79 per cent. w/w sulphuric acid during their study of the carbazole reaction. Further carbohydrates were studied by Ikawa and Niemann,[2,3] who showed the possibility of making use of the ultra-violet absorption spectra of sulphuric acid solutions of the saccharides for analytical purposes. The nature of the reaction between sulphuric acid and carbohydrates has been further investigated by Love[4] and more extensively by Rice and Fishbein[5,6] and Rice.[7]

The object of the work described in this paper was to formulate a rapid method that is suitable for the determination of several different sugars and uronic acids, after each has been obtained in a single pure solution. Previously, a combination of several methods was required to determine a series of aldo- and keto-hexoses, pentoses and uronic acids. The use of concentrated sulphuric acid alone, as a convenient reagent, has been successfully studied, and a suitable procedure has been developed.

METHOD

APPARATUS—

The reaction is carried out in hard-glass test-tubes, 150 mm × 15 mm, lightly closed with a small test-tube, 50 mm × 12·5 mm, in the mouth of each to act as condensers during the heating and to exclude dust particles. Twenty-four tubes are accommodated in a carrier to allow easy and rapid change from the boiling-water bath to cold water.

The absorption spectra and optical densities of the solutions were measured in 1-cm cells with a Unicam SP500 spectrophotometer.

REAGENT—

Sulphuric acid, 98 per cent.—Analytical-reagent grade.

PROCEDURE—

Add 6-ml portions of 98 per cent. sulphuric acid from a burette to the test-tubes, and thoroughly chill in an ice-water bath. Place 1-ml layers of the aqueous carbohydrate solutions, containing up to 100 μg, on the acid from a pipette, and then thoroughly mix by stirring with a glass rod while the tubes remain in the cooling bath. Heat the resulting solutions containing 90 per cent. w/w of sulphuric acid by immersing the tubes for exactly 5 minutes (30 minutes for glucuronolactone) in a bath of rapidly boiling water, and then cool to room temperature in cold water. Include a reagent blank containing 1 ml of water instead of carbohydrate solution with each set of tubes.

Measure the optical densities of the solutions at the appropriate wavelength of maximum absorption for the sugar or uronic acid, namely, arabinose and ribose at 287 mμ, glucuronolactone at 295 mμ, galacturonic acid at 301 mμ, xylose at 316 mμ and fructose, galactose, glucose, mannose and sucrose at 322 mμ.

Experimental

Spectra of reaction products in 90 per cent. sulphuric acid—

The hexoses, fructose, galactose, glucose and mannose, and the disaccharide sucrose, exhibit an absorption maximum at 322 mμ and another less intense peak at 257 mμ. Xylose is similar, although the peak of maximum absorption is at 316 mμ. Ribose and arabinose have maxima at 287 and 316 mμ, the former being of greater intensity for ribose, but of almost equal intensity for arabinose. The uronic acids, under similar conditions, have only a single absorption peak; glucuronolactone at 295 mμ and galacturonic acid at 301 mμ.

The ultra-violet absorption curves for these carbohydrates are shown in Figs. 1 and 2, and it can be seen that the optical density at the wavelength of maximum absorption varies for equal concentrations of the different carbohydrates.

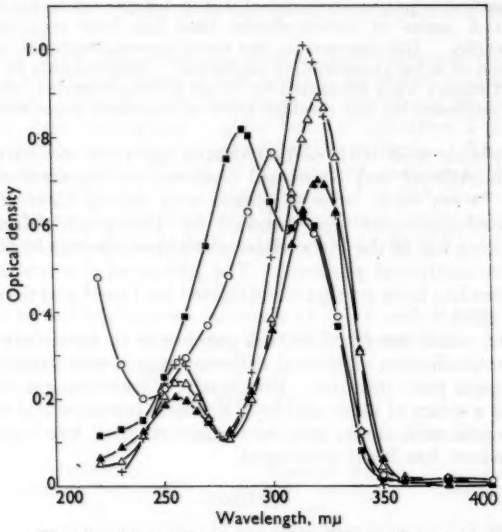

Fig. 1. Absorption spectra of the reaction products formed after heating with sulphuric acid for 5 minutes at 100° C. ○, 100 μg of galacturonic acid per ml; △, 50 μg of fructose per ml; ▲, 50 μg of galactose per ml; ■, 100 μg of ribose per ml; +, 50 μg of xylose per ml

Time of heating—

The determination of the optimum time of heating was made with solutions of sugars and uronic acids in 90 per cent. w/w sulphuric acid (see Fig. 3). The sugars used in the investigations were either the analytical-reagent grades or the laboratory-reagent grades (obtained from the British Drug Houses Ltd. and L. Light & Co. Ltd.), dried *in vacuo* over silica gel. Maximum absorption occurred after heating for 2 to 5 minutes and it decreased slowly with further heating, except with glucuronolactone, when maximum absorption occurred after heating for 30 minutes and thereafter remained practically constant. As any slight variation in the period of heating around 5 minutes has a negligible effect on the measured optical density, 5 minutes were taken as the optimum time of heating for all the carbohydrates studied except glucuronolactone. For this, a time of heating of 30 minutes is required for maximum sensitivity of the method, but a shorter period can be used, as the curve of optical density against lactone concentration is linear after heating for only 5 minutes.

It was also observed that fructose exhibited the same maximum optical-density value if kept for 30 minutes at 20° C as that found after heating for 5 minutes at 100° C, which provides an alternative procedure for this sugar if preferred. The similar behaviour of fructose in 79 per cent. sulphuric acid has been noted by Ikawa and Niemann.[2]

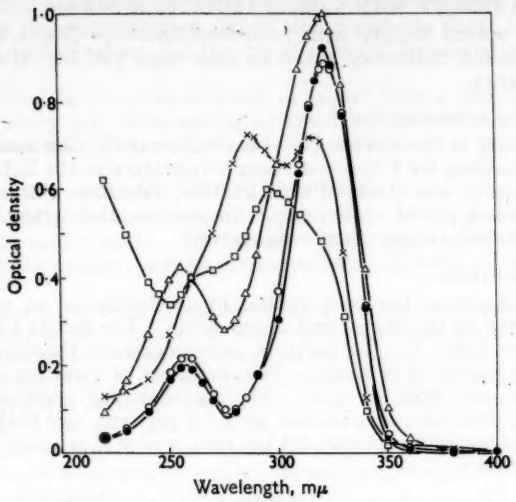

Fig. 2. Absorption spectra of the reaction products formed after heating with sulphuric acid for 5 minutes at 100° C. ○, 50 μg of sucrose per ml; △, 100 μg of mannose per ml; □, 100 μg of glucuronolactone per ml; ●, 50 μg of glucose per ml; ×, 100 μg of arabinose per ml

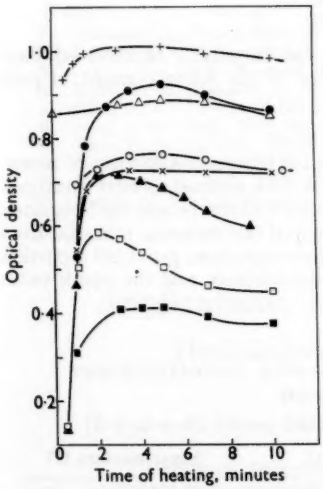

Fig. 3. Change in optical density with time of heating the carbohydrate solutions with sulphuric acid at 100° C. ○, 100 μg of galacturonic acid per ml; △, 50 μg of fructose per ml; □, 100 μg of glucuronolactone per ml; ●, 50 μg of glucose per ml; ▲, 50 μg of galactose per ml; ■, 50 μg of ribose per ml; +, 50 μg of of xylose per ml; ×, 100 μg of arabinose per ml

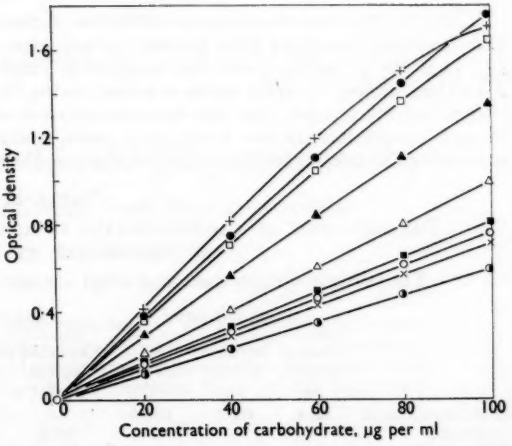

Fig. 4. Change in optical density with concentration of carbohydrate. Solutions were heated with sulphuric acid in accordance with the proposed procedure. ○, galacturonic acid; △, mannose; □, fructose; ●, glucose; ▲, galactose; ■, ribose; ◑, glucuronolactone; +, xylose; ×, arabinose

CHANGE IN OPTICAL DENSITY WITH CONCENTRATION OF SUGARS—

The change in optical density with concentration obeys Beer's law for most sugars, and the use of a standard calibration graph for each sugar (see Fig. 4) overcomes any slight deviation from linearity.

STABILITY OF REACTION PRODUCTS—

The optical density at the wavelength of maximum absorption remains unaltered if the test-tubes are left standing for 3 hours at room temperature in the light, and no significant change in optical density was observed with fructose, galactose, glucose, galacturonic acid, ribose and xylose over a period of 24 hours. In practice, the optical density is measured shortly after the solution attains room temperature.

ACCURACY OF THE METHOD—

The procedure described has been applied in quadruplicate, at each of five different concentrations, to each of the sugars and uronic acids. The results for glucose, which are typical, are shown in Table I. The variance is homogeneous through the range of 20 to 100 μg ($x^2 = 1{\cdot}50$, 4 degrees of freedom). The coefficient of variation at 20 μg was 1·9 per cent. and at 100 μg only 0·35 per cent. The corresponding coefficients of variation at 20 μg and 100 μg, respectively, of arabinose were 1·3 per cent. and 0·45 per cent., fructose, 2·4 per cent. and 0·52 per cent., sucrose, 3·4 per cent. and 0·77 per cent. and xylose, 1·1 per cent. and 0·27 per cent.

TABLE I

ACCURACY OF THE DETERMINATION OF GLUCOSE BY THE METHOD

Glucose concentration, μg per ml	Mean optical density	Range of optical densities	Standard deviation
20	0·379	0·372 to 0·389	0·0051
40	0·750	0·746 to 0·753	
60	1·111	1·105 to 1·114	
80	1·454	1·445 to 1·459	
100	1·764	1·755 to 1·770	

This replication and the fact that the standard curves can be readily reproduced show that the method can be used to give reliable determinations of the sugar content of pure solutions.

APPLICATIONS OF THE METHOD

The method has its primary use in the determination of microgram amounts of mono- and disaccharides eluted from chromatograms after separation with normal solvent mixtures, *e.g.*, as in my experience with the analysis of plant carbohydrate extracts and hydrolysates. A low and almost constant value is found, owing to the elution of chromogenic material from chromatography paper, but this does not interfere with the determination, provided a portion of each chromatogram free from sugar spots is eluted and determined, and the blank value so obtained is subtracted from that of the carbohydrates.

TABLE II

THE RECOVERY OF CARBOHYDRATES SEPARATED BY PAPER CHROMATOGRAPHY AND DETERMINED BY THE METHOD

The solvent system used was ethyl acetate - acetic acid - water (3 + 1 + 3)

	Sugar mixture I			Sugar mixture II			Sugar mixture III		
	Amount applied, μg	Amount found, μg	Recovery, %	Amount applied, μg	Amount found, μg	Recovery, %	Amount applied, μg	Amount found, μg	Recovery, %
Galacturonic acid	62·4	63·4	101·6	—	—	—	—	—	—
Galactose ..	—	—	—	62·5	60·8	97·3	—	—	—
Glucose	—	—	—	—	—	—	62·4	63·0	101·0
Fructose	—	—	—	62·4	62·2	99·7	—	—	—
Arabinose ..	62·3	61·4	98·6	—	—	—	—	—	—
Xylose	—	—	—	—	—	—	62·6	64·3	102·7
Ribose	62·4	62·4	100·0	60·0	59·0	98·3	60·0	61·2	102·0

Any traces of ethyl acetate, acetic acid, pyridine or benzene from developing solvents, which may remain on the chromatogram and be eluted with the sugar, have been found not to interfere with the determination, but the use of solvents containing *n*-butyl alcohol leads to erroneous results.

In experiments to determine the recovery of sugars from a known mixture separated by paper chromatography and subsequently analysed by the method, the recovery ranged from 97·3 to 102·7 per cent. The results in Table II show that the accuracy is satisfactory.

Discussion of results

The method provides an accurate and rapid means of determining aldo- and keto-hexoses, pentoses and uronic acids. The reaction conditions are similar for all the sugars and uronic acids, but the optical densities are measured at different wavelengths in the ultra-violet spectrum.

As the only reagent required is sulphuric acid, the frequent preparation of unstable colour-forming reagents as, for example, is found to be necessary for anthrone,[8] orcinol[9] and *o*-aminodiphenyl,[10] is avoided. The chromogenic compounds formed are unusually stable and possess definite absorption peaks, and the standard curves, which obey Beer's law within the range of concentrations normally encountered, can be readily reproduced, and only one curve need be prepared for each carbohydrate.

Experiments have shown that the reproducibility of readings and the recovery of a mixture of carbohydrates separated by paper chromatography and subsequently determined by the method are satisfactory.

The method can be used for the determination, by one simple procedure once they have been separated, of each carbohydrate present in plant hydrolysates, whereas hitherto a combination of methods has been necessary.

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Applications of Gas-Liquid Chromatography

The Examination of Solvents from Plastic Adhesives

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Details of the methods that have been used in the examination of mixed solvents from plastic adhesives, spray laquers, etc., are given. The isolation of the solvent, its gas-liquid chromatographic separation on polar and non-polar columns and the infra-red and chemical tests on the separated products are described.

In the analytical examination of adhesives, plastic paints, etc., containing various mixed solvents, it is often necessary to express opinions on the composition of the solvent mixture that has been used. Within the past 2 years we have examined a large number of materials, for example, adhesives for bonding plastics to plastics, plastics to metal and plastics to glass,

as well as plastic spraying lacquers, polymer coating solutions and inks suitable for printing on plastic materials.

We have found gas - liquid chromatography to be of great value in the examination of these preparations and the purpose of this paper is to give details of the methods that we have found to be most useful.

It is first necessary to isolate the solvent mixture in a clean condition from the sample under test. The following method has given excellent results in the analysis of a varied range of preparations.

METHOD OF ISOLATING THE SOLVENT MIXTURE FROM THE COMPOSITION UNDER TEST—

An H-tube is constructed having the dimensions shown in Fig. 1. It is a modification of the vacuum-depolymerisation apparatus originally used by Haslam and Soppet.[1] Approximately 5 g of the composition are introduced into the bottom of tube A. This tube is then placed in a solid carbon dioxide - methanol bath at −80° C and the open end, B, is then sealed in a flame. The open end, C, is now attached to a vacuum-pump and the apparatus is evacuated, tube A being kept at −80° C; with the vacuum pump still running the apparatus is then sealed at the constriction, D, by means of a hand torch.

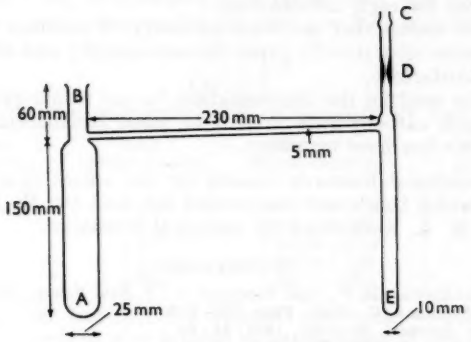


Fig. 1. Modified vacuum-depolymerisation apparatus

Tube A is now removed from the solid carbon dioxide - methanol bath and is replaced by tube E. As tube A gradually attains room temperature, the solvent tends to be volatilised from A to E. This transfer is assisted by warming tube A in a heated water bath or even in a heated oil-bath if the solvent is not readily volatilised. It is often useful to allow this recovery process to proceed overnight. The dimensions of tube A are made purposely large, as some compositions tend to froth during this recovery process.

When the material in A is observed to be "dry," the seal of the apparatus is broken at D and tube E is cut off below the connecting tube.

The mixed solvent in a clean condition is now ready for the preliminary gas - liquid chromatographic test.

It has been reported that some workers introduce the composition directly on to an asbestos pad at the top of a gas - liquid chromatographic column and allow the solvent to evaporate in the gas stream. Apart from the difficulty of introducing such viscous samples by syringe, our experience is that the drops of the composition "harden" on the outside and trap some solvent on the inside. The result is that the solvent dries out slowly and gives a trace that is not a chromatogram. Alternatively, if a high-temperature vaporiser is used, there is a tendency with some preparations to get a chromatogram of the solvent *plus* depolymerisation products. We believe that our method, although more time-consuming, is to be preferred.

PRELIMINARY GAS - LIQUID CHROMATOGRAPHIC TEST—

The purpose of this test is to obtain preliminary information about the general complexity of the mixed solvent.

The gas - liquid chromatographic test is carried out on 1 drop of the isolated solvent; the column is 6 feet long, of ¼-inch nominal bore and packed with 30 per cent. w/w of dinonyl phthalate on Celite 545. The Celite 545 is graded by elutriation in the manner described by James and Martin.[2] The temperature of the column is maintained at 100° C.

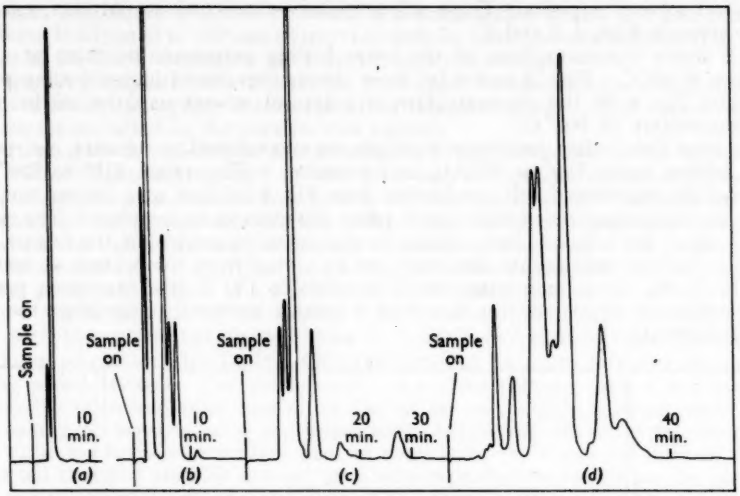


Fig. 2. Gas - liquid chromatograms on a 6-foot column of 30 per cent. w/w of dinonyl phthalate on Celite 545 at 50° C. (*a*) Light petroleum, boiling range below 40° C. (*b*) Light petroleum, boiling range 40° to 60° C. (*c*) Light petroleum, boiling range 60° to 80° C. (*d*) Light petroleum, boiling range 80° to 100° C

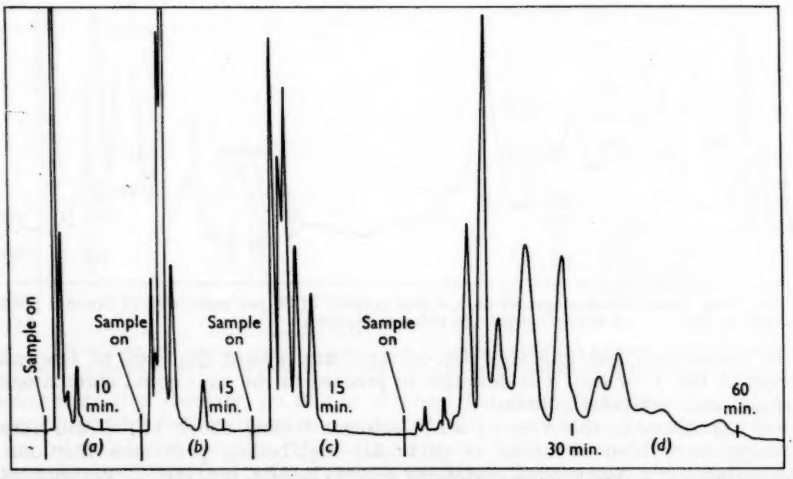


Fig. 3. Gas - liquid chromatograms on a 6-foot column of 30 per cent. w/w of dinonyl phthalate on Celite 545 at 100° C. (*a*) Light petroleum, boiling range 60° to 80° C. (*b*) Light petroleum, boiling range 80° to 100° C. (*c*) Light petroleum, boiling range 100° to 120° C. (*d*) Light petroleum, boiling range above 120° C

The exit pressure is adjusted to 150 mm of mercury and, with a rate of flow of 2·0 litres of nitrogen per hour, the pressure drop along the length of the column is approximately 450 mm of mercury. A katharometer, at room temperature, is used as sensing mechanism.

Visual examination of the chromatogram will quickly indicate the general boiling range of the solvent mixture. If components are present that are rapidly eluted from the column at 100° C, it is desirable to repeat this preliminary separation, but at a column temperature of 50° C.

This test is particularly valuable for ascertaining the presence or absence of petroleum fractions and solvent naphtha. These solvents show characteristic patterns, examples of which are given in Figs. 2, 3 and 4.

Fig. 2 shows chromatograms of the lower boiling petroleum fractions at a column temperature of 50° C. Figs. 3 and 4 (*a*) show chromatograms of higher boiling petroleum fractions and Fig. 4 (*b*) the chromatogram of a typical solvent naphtha carried out at a column temperature of 100° C.

If the very high-boiling petroleum fractions are encountered as solvents, *e.g.*, petroleum distillate, boiling range 190° to 275° C, and kerosine, boiling range 210° to 250° C, some difficulty will be experienced. It can be seen from Fig. 4 (*a*) that, at a column temperature of 100° C, the chromatogram of white spirit takes 100 minutes to complete. The chromatogram of kerosine, for instance, looks similar to that of white spirit, but the former contains many higher boiling constituents that may not be eluted from the column at 100° C. In any case of doubt, the column temperature is raised to 130° C (the maximum permissible with this stationary phase) and the flow rate is greatly increased to facilitate the removal of such constituents.

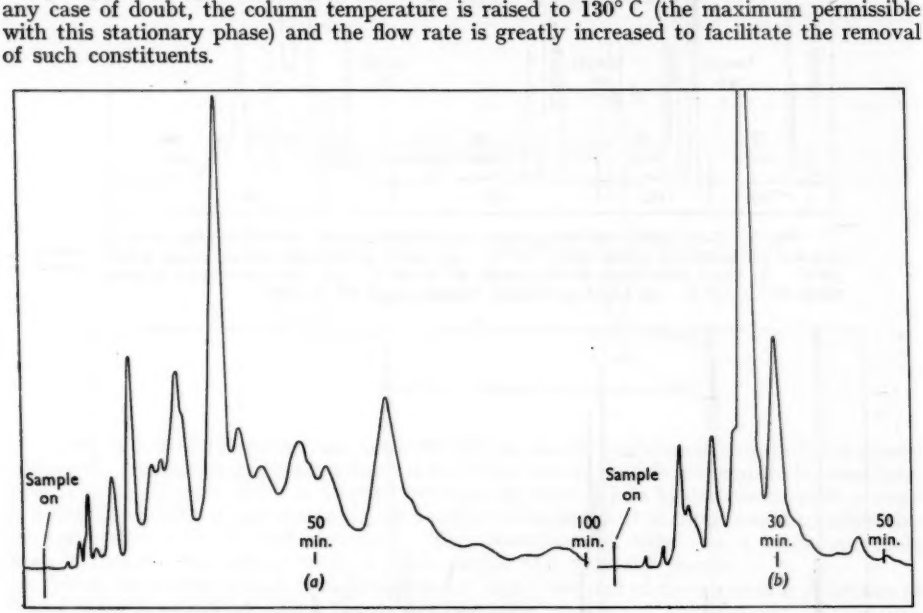


Fig. 4. Gas-liquid chromatograms on a 6-foot column of 30 per cent. w/w of dinonyl phthalate on Celite 545 at 100° C. (*a*) White spirit. (*b*) Solvent naphtha

If the preliminary test indicates that solvents are present that boil at temperatures of the order of 140° C or less, it is desirable to proceed to the gas-liquid chromatographic test on polar and non-polar columns.

In our experience in this type of work, solvents boiling above 140° C will normally, at this stage, have been identified as particular high-boiling petroleum fractions. An occasional instance of a composition containing *n*-butyl lactate, b.p. 188° C, was encountered.

GAS-LIQUID CHROMATOGRAPHIC TESTS ON POLAR AND NON-POLAR COLUMNS—

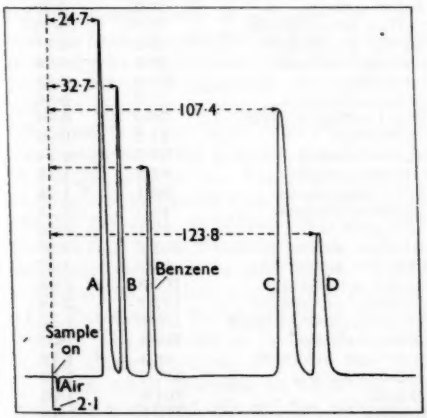
The purpose of this test is to separate, as far as possible, the individual components of a mixed solvent on columns of quite different character, *i.e.*, on (*a*) a non-polar column containing paraffin wax as stationary phase, and (*b*) a polar column containing tritolyl phosphate as stationary phase. The idea underlying this test was first put forward by James and Martin.[3]

Further, in the course of this test the corrected relative retention times of the individual components, *i.e.*, relative to pure benzene, are determined on both columns. The figures are used in the identification of the separated constituents.

The paraffin wax column is 12 feet long, of $\frac{1}{4}$-inch nominal bore and packed with 33·3 per cent. w/w of paraffin wax (congealing point 54° C) on 52 to 60-mesh Johns Manville Silocel C22 firebrick; the column is heated by means of a steam jacket at 100° C. The exit pressure of the column is adjusted to 150 mm of mercury and the inlet pressure controlled so as to give a flow rate of 2·0 litres of nitrogen per hour through the column.

The tritolyl phosphate column is similar to the paraffin wax column, except that 33·3 per cent. w/w of tritolyl phosphate is substituted for the paraffin wax. This column is enclosed in the same steam jacket as the paraffin wax column.

A portion of the sample is first diluted with about one-fifth of its volume of pure benzene and four chromatograms are run, *i.e.*, with and without benzene on each of the columns. Visual examination of the four chromatograms will indicate whether or not benzene is present in the mixed solvent under examination. The corrected relative retention times are now calculated for the individual components separated on each column. The method of calculation is illustrated by the example given in Fig. 5, which represents the chromatogram on the tritolyl phosphate column of a mixed solvent containing ethanol, ethyl acetate, *n*-butyl alcohol, *n*-butyl acetate and added benzene. Comparison of the results obtained on the two columns with the calibration charts shown in Table I will then indicate the composition of most mixed solvents. This Table has been prepared by the examination of known mixtures containing added benzene. Certain solvents, on a given column, were found to have corrected relative retention times similar to that of benzene. Such solvents were examined on their own, pure benzene being run immediately before and after the test substance; the average value for benzene was then used in computing the value for the test substance. It was noted that the alcohols did not give very reproducible results on the paraffin wax column and the values given are the average of several results.



Calculation of corrected relative retention times—

Peak A $\frac{(24{\cdot}7-2{\cdot}1)}{(45{\cdot}5-2{\cdot}1)} = 0{\cdot}52$

Peak B $\frac{(32{\cdot}7-2{\cdot}1)}{(45{\cdot}5-2{\cdot}1)} = 0{\cdot}71$

Peak C $\frac{(107{\cdot}4-2{\cdot}1)}{(45{\cdot}5-2{\cdot}1)} = 2{\cdot}43$

Peak D $\frac{(123{\cdot}8-2{\cdot}1)}{(45{\cdot}5-2{\cdot}1)} = 2{\cdot}80$

Solvent mixture sample identified as—

A = Ethanol
B = Ethyl acetate
C = *n*-Butyl alcohol
D = *n*-Butyl acetate

Fig. 5. Specimen gas-liquid chromatogram of a mixed solvent on a 12-foot column of 33·3 per cent. w/w of tritolyl phosphate on Silocel C22 for determining the corrected relative retention times

Apart from this anomaly, we find in practice that the reproducibility of the test is of the order of ±0·02 to 0·03 units up to a corrected relative retention time of 1·0, ±0·04 to 0·08 units for 1·0 to 3·5 and ±0·1 to 0·15 units for above 3·5. It may be necessary, therefore, that, in order to obtain two peaks in a chromatogram from a mixture of two substances, their corrected relative retention times should differ by as much as 0·08 units up to a corrected relative retention time of 1 and 0·15 to 0·25 units for a corrected relative retention time above 1.

It will be realised from these figures that difficulties may arise because of the overlap of peaks, and for this and other reasons we find it invaluable when there is any doubt to carry out separations on columns with isolation of the separated components and infra-red examination of the separated products. For example, in our experience a mixture of methyl acetate and acetone gives a single peak on both columns, but such a mixture would not

deceive an infra-red spectroscopist. A corresponding instance would be that of a mixture of *m*-xylene and *p*-xylene.

TABLE I

CALCULATED RELATIVE RETENTION TIMES FOR VARIOUS SOLVENTS ON 12-FOOT COLUMNS OF 33·3 PER CENT. OF PARAFFIN WAX ON SILOCEL C22 AND 33·3 PER CENT. OF TRITOLYL PHOSPHATE ON SILOCEL C22

Solvent	Boiling-point, °C	Calculated relative retention time	Solvent	Boiling-point, °C	Calculated relative retention time
Paraffin wax column—			*Tritolyl phosphate column*—		
Acetaldehyde	20·2	0·09	*n*-Pentane	36·1	0·11
Methyl formate	31·5	0·11	Diethyl ether	34·6	0·17
Methanol	64·6	0·13	Acetaldehyde	20·2	0·19
Ethanol	78·3	0·17	Methyl formate	31·5	0·22
Acetone	56·0	0·18	Di*iso*propyl ether	67·5	0·29
Methyl acetate	57·1	0·23	Methanol	64·6	0·36
*iso*Propyl alcohol	82·4	0·23	*iso*Octane	99·2	0·37
Ethyl formate	54·2	0·23	Ethyl formate	54·2	0·38
Allyl alcohol	97·1	0·25	Methyl acetate	57·1	0·40
Diethyl ether	34·6	0·26	*n*-Heptane	98·4	0·42
Methylene dichloride	40·1	0·27	*cyclo*Hexane	80·8	0·45
tert.-Butyl alcohol	82·5	0·28	Acetone	56·0	0·46
n-Pentane	36·1	0·29	Methylene dichloride	40·1	0·47
Vinyl acetate	77·0	0·35	Ethanol	78·3	0·52
n-Propyl alcohol	97·2	0·36	*tert.*-Butyl alcohol	82·5	0·53
Ethyl methyl ketone	80·0	0·40	Ethylidene dichloride	57·3	0·57
Ethyl acetate	77·2	0·43	*iso*Propyl alcohol	82·4	0·58
Ethylidene dichloride	57·3	0·44	Vinyl acetate	77·0	0·59
sec.-Butyl alcohol	99·5	0·50	Methyl*cyclo*hexane	100·8	0·66
Di*iso*propyl ether	67·5	0·52	Ethyl acetate	77·2	0·70
Methyl propionate	79·9	0·54	Carbon tetrachloride	76·7	0·76
*iso*Butyl alcohol	108·1	0·55	Methyl propionate	79·9	0·77
*iso*Propyl acetate	88·9	0·58	*iso*Propyl acetate	88·9	0·79
Chloroform	61·2	0·60	*n*-Octane	125·6	0·85
Tetrahydrofuran	65·0	0·62	Tetrahydrofuran	65·0	0·86
Ethylene dichloride	83·5	0·72	Ethyl methyl ketone	80·0	0·89
Methyl *n*-propyl ketone	102·3	0·81	Chloroform	61·2	0·99
n-Butyl alcohol	118·0	0·83	*sec.*-Butyl alcohol	99·5	1·14
n-Propyl acetate	101·6	0·93	*n*-Propyl alcohol	97·2	1·15
Ethyl propionate	99·1	0·94	Ethyl propionate	99·1	1·24
Carbon tetrachloride	76·7	1·06	Trichloroethylene	86·7	1·26
Methyl *n*-butyrate	102·3	1·09	Allyl alcohol	97·1	1·26
Dioxan	101·4	1·12	*n*-Propyl acetate	101·6	1·35
*cyclo*Hexane	80·8	1·14	Ethylene dichloride	83·5	1·37
Propylene dichloride	96·4	1·17	Methyl *n*-butyrate	102·3	1·46
*iso*Propyl propionate	111·3	1·25	*iso*Propyl propionate	111·3	1·48
*iso*Butyl methyl ketone	116·8	1·28	Methyl *n*-propyl ketone	102·3	1·55
Trichloroethylene	86·7	1·35	*iso*Butyl alcohol	108·1	1·65
*iso*Octane	99·2	1·37	Propylene dichloride	96·4	1·76
n-Heptane	98·4	1·40	*iso*Butyl acetate	117·2	1·93
*iso*Butyl acetate	117·2	1·51	Dioxan	101·4	1·95
Methyl*cyclo*hexane	100·8	1·91	Toluene	110·8	2·10
n-Butyl acetate	126·2	2·13	*iso*Butyl methyl ketone	116·8	2·12
Toluene	110·8	2·32	*n*-Butyl alcohol	118·0	2·41
n-Octane	125·6	3·10	Di-*n*-butyl ether	142·4	2·41
Ethylbenzene	136·2	4·6	*n*-Butyl acetate	126·2	2·78
Di-*n*-butyl ether	142·4	4·8	Ethylbenzene	136·2	4·1
p-Xylene	138·4	5·2	*p*-Xylene	138·4	4·2
m-Xylene	139·3	5·3	*m*-Xylene	139·3	4·4
o-Xylene	144·0	6·1	*o*-Xylene	144·0	5·3

CHROMATOGRAPHIC SEPARATION AND INFRA-RED EXAMINATION OF SEPARATED PRODUCTS—

The great advantage of this method is that, although the column may have to be loaded with a comparatively large amount of sample, and hence there may be a considerable loss in column efficiency, once the products have been separated their infra-red identification is usually unequivocal.

The principle of this method, which involves the trapping of the separated products after passage through the column, has been described previously.[4] In the interval, the trapping system has been modified and reduced in size. Its dimensions are shown in Fig. 6.

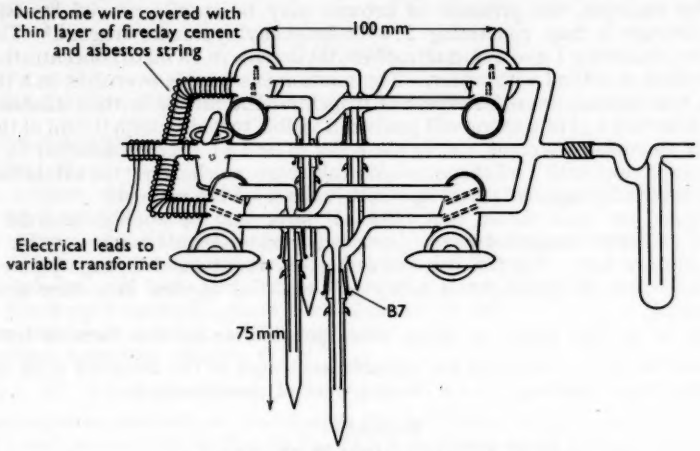

Nichrome wire covered with thin layer of fireclay cement and asbestos string
100 mm
Electrical leads to variable transformer
B7
75 mm

Fig. 6. Modified trapping apparatus

The choice of column, load and conditions of separation in this test are largely governed by the boiling range of the mixture as indicated by the preliminary chromatogram. The column temperature is adjusted so that complete separation between the peaks, with a suitable load, is indicated by the record. It must be borne in mind, however, that it is usually necessary to separate at least 0·05 ml of a particular constituent in order to obtain a satisfactory infra-red spectrum, although work is being carried out to extend the range of the tests so that much smaller amounts of substance can be dealt with. With very volatile constituents it may be necessary to separate rather more than this amount. We have pointed out previously[4] that a single peak in a chromatogram does not always indicate the presence of a single substance. Recently, a spray lacquer was examined; this contained 16 per cent. of poly(vinyl chloride - vinyl acetate) copolymer, 9 per cent. of dioctyl phthalate plasticiser and 75 per cent. of mixed solvent. The preliminary chromatogram of the mixed solvent showed four quite separate peaks. The constituents corresponding to these four peaks were condensed in separate cold traps. Infra-red examination indicated that trap 1 contained a mixture of methyl acetate and acetone (a mixture difficult to resolve on many columns). Trap 2 contained tetrahydrofuran and traps 3 and 4 contained benzene and toluene, respectively. The mixture in fact contained five components, although only four were indicated in the chromatogram.

It is often desirable to ascertain the approximate composition of the solvent mixture and this is carried out by the method described below.

DETERMINATION OF THE APPROXIMATE COMPOSITION OF SOLVENT MIXTURES—

From the chromatograms obtained as described it is usually possible to estimate the approximate composition of an unknown solvent mixture. One or two synthetic mixtures similar to the estimated composition of the sample are examined under the precise conditions of test as with the unknown mixture. The composition of this latter mixture is then deduced by inspection of the chromatograms.

With experience, the accuracy is normally quite adequate for most work of this type. If at any time greater accuracy is required, a calibration for each component with an internal marker is necessary when nitrogen is used as the carrier gas in conjunction with a katharometer detector.

Finally, on occasion, use may be made of chemical tests on separated products.

CHEMICAL TESTS ON SEPARATED PRODUCTS—

In addition to the infra-red identification, it is possible to prepare chemically suitable derivatives of the isolated products. Moreover, certain tests may be made directly on the separated products (diluted with carrier gas) as they leave the gas - liquid chromatographic column. For example, the presence of ketones may be readily proved by bubbling the exit gases through a trap containing 2:4-dinitrophenylhydrazine reagent.[5] This reagent is prepared by dissolving 1 g of 2:4-dinitrophenylhydrazine in 15 ml of concentrated sulphuric acid and diluting to 400 ml with water. The solution is set aside overnight in a refrigerator, after which it is filtered from the excess of reagent. The filtrate is then diluted to 500 ml with water, 0·05 to 0·1 μl of acetone will produce a visible turbidity with 0·5 ml of this reagent. Even smaller amounts of acetone, *i.e.*, of the order of 0·01 μl, may be detected by extraction of the reaction product with 1 ml of spectroscopically pure *cyclo*hexane; the extract is examined spectrophotometrically against a corresponding extract of the reagent.

Then again, we have shown that chemical tests may be applied in order to decide whether, for example, formaldehyde is produced from methanol under specific conditions of chromatographic test. For this test the exit gases were passed through phenylhydrazine reagent, after which the principle of Schryver's test was applied in a very sensitive test for formaldehyde.

It seems to us that there are many other possibilities for this form of test.

We thank Mr. H. A. Willis for his valuable assistance in the infra-red work and Mr. M. Green for his general assistance in the development of these methods.

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The Separation of some Coal-tar Food Colours by Paper Electrophoresis

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The behaviour during electrophoresis on paper of some coal-tar food colours in different electrolytes is described and discussed. Results show that, in suitable electrolytes, it is possible to separate individual colours from mixtures by paper electrophoresis.

LEDERER[1] gives an excellent account of the basic principles and applications of paper electrophoresis, and includes a brief survey of published work on the electrophoresis of dye-stuffs. However, the only work published on the separation of food colours by paper electrophoresis is that of Mori and Kimura[2] and Mori,[3] who have examined the behaviour of colours under different conditions of electrolyte, filter-paper, current and so on, and Williams,[4] who describes the electrophoretic isolation at pH 12 of dye-stuffs from biscuits, jams and cream confectionery.

The chromatographic isolation of food colours has received greater attention, although it is only 6 years since Tilden[5] remarked that, of the many applications of paper partition-chromatography, relatively few dealt with the separation and isolation of dye materials. Many papers have since appeared that describe methods for the chromatographic separation of coal-tar food colours. Some workers have used the column technique,[6,7] but many others have dealt with separations on paper.[8 to 14] Fujii[10] summarises the chromatographic behaviour of no less than ninety-five artificial coal-tar dyes, and Verma and Das[11] have determined the R_F values, in thirteen eluting agents covering a wide pH range from acid to alkaline conditions, of forty-five dyes commonly used in foodstuffs. The identification of colouring matter in specific foodstuffs has been dealt with by some workers, and the range of foodstuffs examined includes fruit squash,[8] caviare,[9] wines, syrups and so on[12] and jelly crystals.[14]

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Of the twenty-six food colours examined by Mori and Kimura[2] and Mori[3] by paper electrophoresis, only three are among the permitted[15] coal-tar food colours, namely, amaranth, tartrazine and indigo carmine. We now describe an extension of the work to an examination of the behaviour of erythrosine BS, tartrazine, indigo carmine, ponceau MX, ponceau 4R and ponceau 3R.

Method

Apparatus—

An E.E.L. (Evans Electroselenium Ltd.) electrophoresis apparatus was used.

Electrolyte solutions—

Acetic acid, N.

Buffer solution, pH 4·0—Add 6·0 ml of 0·1 *N* sodium hydroxide to 750 ml of 0·1 *M* potassium hydrogen phthalate, and dilute to 1·5 litres.

Buffer solution, pH 6·0—Add 85·5 ml of 0·1 *N* sodium hydroxide to 750 ml of 0·1 *M* potassium dihydrogen orthophosphate, and dilute to 1·5 litres.

Buffer solution, pH 8·0—Add 702 ml of 0·1 *N* sodium hydroxide to 750 ml of 0·1 *M* potassium dihydrogen orthophosphate, and dilute to 1·5 litres.

Sodium tetraborate solution, 1 *per cent. w/v.*

Ammonium hydroxide solution, 0·1 N.

Preparation of dye solutions—

Prepare aqueous solutions of erythrosine BS, tartrazine, indigo carmine, ponceau MX, ponceau 4R and ponceau 3R, so that 50 ml of each solution contain 10 mg of the dye. When a mixture is to be used, prepare a solution containing 10 mg of each constituent per 50 ml.

Procedure—

Fill the four compartments of the electrophoresis bath to the same level with the relevant electrolyte solution. Cut a Whatman No. 1 filter-paper strip (5 cm wide) to suitable length (34 cm).

Draw a faint pencil line across the filter-paper strip, 5 cm from one end. Thoroughly moisten the strip with the electrolyte solution in the bath, place it across the "bridge," and apply evenly at the pencil line 0·02 ml of the relevant dye solution, which corresponds to 4 μg of each dye. When there is more than one strip in the bath, take care that all the samples are at the same end. Place the glass cover on the bath and connect to the power unit. Place the polarity switch in the position indicated by the position of the sample, *i.e.*, if the pencil mark is at end A of the bath, place switch in position A also and *vice versa*. This makes the sample end the cathode compartment. (The dyes in each instance migrate towards the anode, although some of those examined by Mori and Kimura[2] migrate towards the cathode, *e.g.*, Bismarck brown and rhodamine 6G.) Allow electrophoresis to take place by switching on the power unit and adjusting the current to a suitable value.

After a suitable time interval, end the experiment by switching off the power unit and disconnecting the electrophoresis bath. Remove the strips and dry them in an oven at 110° C for 5 minutes, the strips being suspended loopwise from a glass rod by means of "bulldog" clips at either end. The electropherogram is then ready for examination.

Results

The dyes were subjected to paper electrophoresis singly and also as constituents of mixtures, the observed migration distances being reproducible. Table I shows the distances migrated by the dyes, both singly and as constituents of mixtures. Migration distances of less than 2 mm are shown as zero.

Discussion of results

Table I shows a number of peculiarities and trends that call for comment.

Migration distances in a given time for the dyes in the nearly neutral electrolytes, *i.e.*, buffer solutions pH 6·0 and 8·0, are relatively small when compared with the much greater distances observed under more acid or alkaline conditions. This would suggest that separations can be carried out more effectively under distinctly acid or alkaline conditions. This

is found not to be so, as the advantage gained by greater migration distances is lost because of the considerable and often rapid tailing and fading that occurs under these extreme conditions. It is worthy of note, however, that erythrosine BS, tartrazine and indigo carmine have been separated in 15 minutes with *N* acetic acid as electrolyte, the respective migration distances being 0, 16 and 7 mm at a current density of 0·6 mA per strip, tailing and fading being only slight.

TABLE I

MIGRATION DISTANCES OF DYES

Electrolyte	Current density, mA per 5 cm	Time, hours	Migration distance of— erythrosine BS, mm	tartrazine, mm	indigo carmine, mm	ponceau MX, mm	ponceau 4R, mm	ponceau 3R, mm
Single dyes—								
Acetic acid, *N*	0·6	2	0	130*	52†	—	—	—
	1·7	2	—	—	—	~9‡	37*	20*
Buffer solution, pH 4·0	1·7	1·75	0	74*	35§	—	—	—
Buffer solution, pH 6·0	1·7	2	0	23	9	0	26	0
Buffer solution, pH 8·0	2·0	2	0	15	8	0	30	0
Sodium tetraborate solution, 1 per cent.	2·0	1·5	9	103†	38*	0, 16‖	100	11
Ammonium hydroxide, 0·1 *N*	1·7	2	0	83*	10*	—	—	—
Dye mixtures (each horizontal line corresponds to a mixture)—								
Acetic acid, *N*	0·6	0·25	0	16	7	—	—	—
	0·6	1·5	0	90*	40†	—	—	—
Buffer solution, pH 4·0	1·7	1·75	0	70*	32†	—	—	—
Buffer solution, pH 6·0	1·7	2	0	24	10	—	—	—
	1·7	2	0	23	8	0	27	0
Buffer solution, pH 8·0	2·0	2	0	18	7	—	—	—
	2·0	2	0	18	7	0	30	0
Sodium tetraborate solution, 1 per cent.	2·0	1·5	—	—	—	0, 17‖	75	9
	2·0	1·5	10	85†	—‡	0, 16‖	80	10
Ammonium hydroxide, 0·1 *N*	1·7	2	0	83*	9‡	—	—	—

* Tailing and fading. † Tailing. ‡ Fading.
§ Slight tailing and fading. ‖ Two bands, but the band at 0 mm is faint.

In experiments in which electrophoresis was carried out for different time intervals, the other conditions being kept constant, an approximately linear relationship between migration distance and time was observed (compare Mori[3]), *e.g.*, for tartrazine and indigo carmine in *N* acetic acid for 15 and 90 minutes, the respective migration distances were 16 and 90 mm for tartrazine and 7 and 40 mm for indigo carmine.

It is often observed that, when dyes are present in a mixture, they exert a "dragging" effect on one another. This is particularly marked with tartrazine and ponceau 4R in 1 per cent. sodium tetraborate solution, the migration distance of tartrazine being reduced from 103 mm for the single dye to 85 mm for the dye present in a mixture; corresponding figures for ponceau 4R are 100 and 80 mm.

The four red dyes, erythrosine BS, ponceau MX, ponceau 4R and ponceau 3R, were chosen for this investigation because of their similarity in colour and, in so far as the ponceaus are concerned, their similarity in structure. As is to be expected, and as the results show, their separation by paper electrophoresis is more difficult than for the other dyes. Ponceau 4R, however, presents little difficulty, as it migrates readily in every instance. Except in 1 per cent. sodium tetraborate solution, erythrosine BS, ponceau MX and ponceau 3R did

not separate, although separation would be possible in *N* acetic acid, except for the considerable tailing and fading that occurs in this electrolyte. When the six dyes are present in one mixture, separation into four distinct bands takes place in 1 per cent. sodium tetraborate solution, a fifth band of indigo carmine being completely faded. Two dyes that cannot be separated in this electrolyte are erythrosine BS and ponceau 3R.

The difficulty in separating ponceau MX and ponceau 3R in many electrolytes can probably be attributed to the similarity in structure (ponceau 3R has one extra methyl group). The observations of Anderson and Martin,[14] who examined the effect of substituents on the R_F values when dye-stuffs were chromatographically extracted with *iso*butyl alcohol saturated with 2 *N* hydrochloric acid, are of interest in this connection. They report that the R_F value of a mono-azo dye-stuff is not influenced by substituent groups such as methyl, but is increased by a decrease in the number of sulphonic acid groups. These trends cannot hold for paper electrophoresis, as it is possible to separate ponceau MX and ponceau 3R in both *N* acetic acid and 1 per cent. sodium tetraborate solution. Further, ponceau MX, a mixture of dyes with methyl groups in different positions, has been separated into two bands in 1 per cent. sodium tetraborate solution. Also, the effect of the sulphonic acid group appears to be the reverse of that for chromatographic separation, *i.e.*, an increase in the number of sulphonic acid groups increases the migration distance, as illustrated by ponceau 4R (three sulphonic acid groups) and ponceau MX and ponceau 3R (two sulphonic acid groups).

Conclusions

With a suitable choice of electrolytes it is possible to separate food-colouring materials by paper electrophoresis. Distinct separations of dyes from mixtures are possible, as long as there is a difference of 3 to 4 mm between the migration distance of each dye. This difference must of necessity be greater when tailing occurs. Two to three hours are usually sufficient to isolate the components into distinct zones, which can be cut out, the dyes eluted with distilled water and absorption spectra recorded. With suitable colour filters, which are available from the manufacturers, the E.E.L. scanner can also be used for quantitative evaluation.

We thank Solmedia Ltd. for a gift of ponceau MX, ponceau 4R and ponceau 3R.

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Atomic-absorption Spectrophotometry with Special Reference to the Determination of Magnesium

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The determination of magnesium by atomic absorption is examined in detail. The apparatus and method are described, and the results are discussed with regard to reproducibility, accuracy and sensitivity. It is shown that, by this method, reliable magnesium determinations can be made with the same ease and rapidity as flame-photometric determinations of sodium and potassium.

THE possible use of atomic-absorption spectra for general analytical purposes was first realised by Walsh, who, in 1955,[1] considered the theoretical and experimental problems involved and concluded that the method showed considerable promise and could have several advantages over emission methods. In a later paper,[2] an experimental atomic-absorption spectrophotometer was described and results were given for a number of elements. In this laboratory, the determination of elements of agricultural interest by the method is being studied and the results obtained for magnesium are presented here.

In the analysis of plant material, soil extracts and so on, magnesium, of the four major cations present, is the most difficult to determine. The other three, sodium, potassium and calcium, can be readily determined with speed and accuracy by flame-photometric methods, and for some years in this laboratory a simple triple-beam filter flame photometer has been used for this purpose. As magnesium emission is weak compared with the flame background, the method cannot be applied to the determination of this element. Reasonable results can be obtained by using a monochromator to isolate the magnesium line, but, even when this is done, the necessity of backing off a large background reading and the fact that the emission - concentration curve is flattened by self-absorption reduce the sensitivity and precision.

The fact that magnesium emits weakly and shows strong self-absorption made it appear that the atomic-absorption method would be particularly suitable for its determination. That this is so has been fully confirmed by the results reported later.

Experimental

Briefly, the basis of the method is the measurement of the light absorbed at the wavelength of the resonance line by the unexcited atoms of the element. This measurement is made by spraying the sample into a flame to provide a reproducible and clearly defined cloud of atoms and by using as the light source a lamp that emits the line spectra of the element to be determined. The apparatus used is shown in Fig. 1.

Light source—

Emission from the light source should be steady, to avoid the necessity for double-beam operation, and should be intense in relation to the emission from the flame, to avoid having to eliminate the latter. Further, the lines should be sharp and narrower than the absorption line in the flame so that the peak absorption can be measured. For magnesium, these requirements are met by the magnesium - aluminium hollow-cathode lamp manufactured by Hilger and Watts.

This lamp, which has a cathode of duralumin, operates at currents up to 70 mA, with a voltage drop across the lamp of about 260 volts. About 500 volts are required to start the discharge and usually a power supply that delivers a somewhat greater voltage is used with a series resistor to control the current. A full description of the operation of a hollow-cathode lamp with an iron cathode has been published by Crosswhite, Dieke and Legagneur.[3] The magnesium - aluminium lamp, however, differs from the iron lamp in that the emission does not reach maximum intensity immediately after the lamp is switched on, but requires a warming-up period of at least 30 minutes. During this time, the intensity of the aluminium spectrum is reasonably constant, but that of the magnesium spectrum increases greatly. Similarly, when the current through the lamp is changed, the same interval should be allowed for the intensity to reach a steady value.

FIG. 1. Apparatus for determining magnesium by atomic absorption

[To face p. 466

Both the intensity of emission and the width of the emitted line vary with the current. The intensities of a number of lines as a function of the current are shown in Fig. 2.

The curves in Fig. 2 show that the intensities of both the aluminium and magnesium lines increase more rapidly than the current, and, to keep the intensity of the magnesium line at 2852 A to within ± 1 per cent. of the value at 60 mA, the current must be controlled to within $\pm 0{\cdot}1$ mA.

The effect on the calibration curve of the widening of the magnesium line at 2852 A, which occurs as the current is increased, is shown by curves A, B, C and D in Fig. 4, p. 468, and will be referred to later. On this account also, close control of the current is necessary.

It was found to be convenient to operate the lamp from an electronically regulated power supply of conventional design, which delivered a regulated voltage of 1150 volts. This voltage, which is also used to operate the photomultiplier tube referred to later, is applied to the hollow-cathode lamp as shown in Fig. 3. In this circuit, the 807 valve tends to maintain a constant anode current, which compensates changes in the resistance of the hollow-cathode lamp. The current through the lamp can be set to any desired value between 35 and 70 mA by means of resistor R_3.

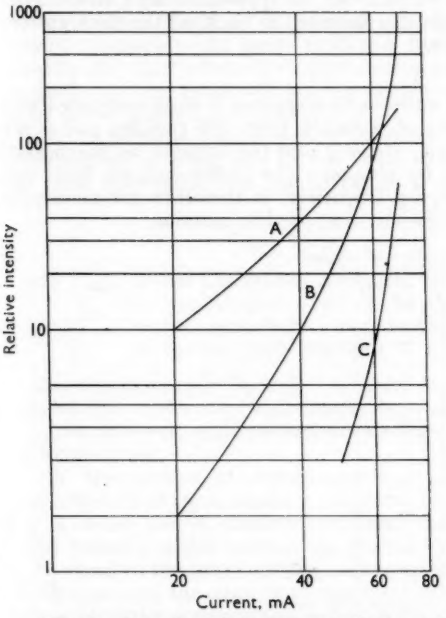
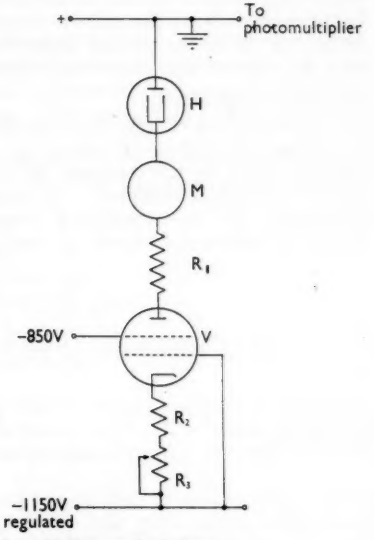


Fig. 2. Intensity of the hollow-cathode spectrum as a function of the current: curve A, Al I 3093 A line; curve B, Mg I 2852 A line; curve C, Mg II 2796 A line



H = Hollow-cathode lamp
M = 0 to 100 mA milliameter
R_1 = 9500-ohm 50-watt wire-wound resistor
R_2 = 200-ohm 2-watt wire-wound resistor
R_3 = 500-ohm 5-watt wire-wound resistor
V = 807 valve

Fig. 3. Current-control circuit for hollow-cathode lamp

BURNER AND ATOMISER—

The burner shown in Fig. 1 is the standard Lundegårdh type, which burns an air - acetylene mixture supplied at the rate of 1·1 litres of acetylene and 8 litres of air per minute. The atomiser (not shown in Fig. 1) is basically a Lundegårdh atomiser, but has been modified to suck the solution up from a beaker to avoid the usual procedure of dismantling the spray chamber when solutions are changed. About 7·0 ml of solution are used per minute, of which about 0·12 ml enters the flame. Other burners have been used with the same atomiser and will be discussed later.

After the burner, a diaphragm with a $\frac{1}{4}$-inch diameter opening prevents entry into the spectrograph of light from the blue cone of the flame.

The height of the burner is adjusted so that the beam of light from the hollow-cathode lamp, which reaches the spectrograph through the diaphragm, passes through the flame about ½ inch above the top of the burner.

The hollow-cathode lamp and the burner are placed reasonably close together and about 18 inches from the slit of the spectrograph. This is not at all critical, but the light that enters the spectrograph from the flame is reduced in relation to that from the lamp as the distance from the spectrograph is increased.

SPECTROGRAPH—

The spectrograph used is a Hilger medium-quartz instrument with an exit slit and a photomultiplier (R.C.A. IP28), positioned to intercept the magnesium line at 2852 A, mounted on a spare plate holder. As the spectrum of the lamp is practically free from background and as there are no lines close to the magnesium line at 2852 A, except a very weak line at 2856 A, reasonably wide entrance and exit slits can be used (about 0·5 mm), thus permitting the plate holder carrying the exit slit and the photomultiplier to be removed and replaced without fine adjustment.

The anode current of the IP28 is measured by a Cambridge galvanometer (450 ohms), which is provided with a variable shunt to control its sensitivity. By means of a 1·5-volt battery and a variable resistor, a small direct current can be applied to the galvanometer in opposition to the current from the photomultiplier in order to back off the dark current and the current due to emission from the flame.

GENERAL OPERATION—

The emission of light from the flame at the wavelength measured is weak compared with that from the hollow-cathode lamp. It consists of radiation from OH radicles and is unaffected either by the presence of cations or anions sprayed into the flame or by magnesium in the amounts considered. Its removal, *e.g.*, by chopping the hollow-cathode light and amplifying the photomultiplier output at chopping frequency, is therefore not necessary, and it is sufficient to back off electrically the current due to this emission.

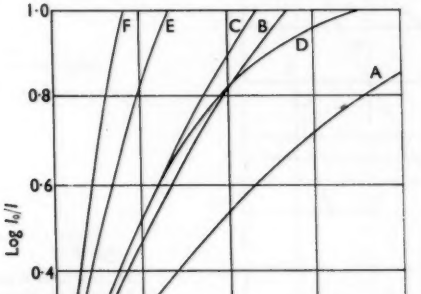


Fig. 4. Calibration curves for magnesium at different current supplies to the hollow-cathode lamp and different flame widths: curve A, 70 mA, 2·1 cm; curve B, 60 mA, 2·1 cm; curve C, 50 mA, 2·1 cm; curve D, 40 mA, 2·1 cm; curve E, 60 mA, 4·8 cm; curve F, 60 mA, 7·5 cm

The procedure for measuring the absorption is therefore as follows. After at least 30 minutes for the hollow-cathode lamp to attain a steady emission and for the photomultiplier to fatigue, distilled water is sprayed into the flame and, with a shutter in front of the

lamp, the small galvanometer reading, about 5 divisions, due to the photomultiplier dark current and flame emission is backed off electrically. The shutter is then opened and the galvanometer adjusted to the full-scale reading with the variable shunt. Distilled water is then replaced by the solution to be analysed and the galvanometer reading is taken. After ten or twelve solutions have been analysed, the zero and full-scale readings are again checked with distilled water in the flame. The zero reading rarely alters, but the full-scale reading may change slightly, owing to photomultiplier fatigue.

Results and discussion

Sensitivity—

The extent to which the measured absorption approaches the peak value will depend on (*a*) the ability of the monochromator to resolve the line being measured from the other radiation of the lamp, and (*b*) the width of this line in relation to that of the absorption line in the flame. As the spectrum emitted by the lamp is practically free from background, the resolution of the monochromator is not of great importance in the present instance, provided that the magnesium line at 2852 A is separated from other lines. The effect of reducing the width of the hollow-cathode line by decreasing the current is shown in Fig. 4, from which it can be seen that, down to 50 mA, an increase in absorption is obtained. At the same time, of course, the intensity of the lamp is reduced, and it was found to be convenient to operate the lamp at 60 mA for routine analytical work. An improvement in linearity also results from the narrower line, although some curvature always remains, caused, no doubt, by the finite width of the hollow-cathode line. The greatly increased curvature at 40 mA is difficult to explain.

When pressure broadening in the flame is ignored and the shape of the absorption line is considered as being due to the Doppler broadening only, then the relationship between the peak absorption and concentration is given by[4]—

$$K_{\max.} = \frac{2\lambda^2}{D_\lambda}\sqrt{\frac{\ln 2}{\pi}} \cdot \frac{\pi e^2}{mc^2} \cdot Nf$$

where $K_{\max.}$ = the absorption coefficient at the centre of the line,
D_λ = the Doppler width of the line,
N = the number of atoms capable of absorbing at wavelength λ, and
f = the oscillator strength of the line.

In this equation, only D_λ and N are subject to experimental conditions. D_λ for a particular element line alters only with temperature and is proportional to $T^{\frac{1}{2}}$. The effect of this on $K_{\max.}$ is, however, unimportant in practice, as it is overshadowed by the dependence of N on temperature.

N, the number of atoms capable of absorbing, is proportional to the product of the concentration of these atoms in the flame and the length of the light path through the flame. Fig. 4 shows curves obtained by using three burners of different length. When the two larger burners, which were of the fish-tail type, were used, the length of the flame appeared to be equal to the length of the burner opening. The smallest burner was the usual Lundegårdh type, and the flame path was somewhat longer than the burner opening (about 2·4 cm). The sensitivities obtained can be seen to be approximately proportional to the length of flame. There is, of course, a limit to the increase in sensitivity that can be obtained in this way. The burner opening cannot be decreased in width unduly or it will become blocked by salt deposits. As the length increases, therefore, the area of the opening increases and eventually the flame will become unstable and flash back.

As the number of excited atoms is always a very small fraction of the total, the number of unexcited atoms is virtually equal to the total number of atoms, and the concentration of these in the flame will depend on two factors. The first is the efficiency of the atomiser as judged by the amount of spray, sufficiently finely divided to be completely volatile, that is introduced into the flame per unit volume of air. It is of interest to note that, in emission flame photometry, if the sensitivity is expressed as the concentration required to give the full-scale reading, it is possible to camouflage a poor atomiser by using an optical system of greater light-gathering power or a more sensitive galvanometer or photocell. Such devices are, however, of no avail in the measurement of absorption, which therefore provides a direct test of atomiser efficiency.

The second factor on which the concentration of atoms in the flame depends is obviously the dissociation of the magnesium compounds and this will depend on the flame temperature. From the work of Huldt and Lagerquist,[5] who determined the concentration of free magnesium atoms in a flame similar to that used in this instance, with a temperature of 2410° K, it appears that only a small proportion of the total magnesium (about 1·5 per cent.) is dissociated into atoms, the rest presumably existing as magnesium oxide. If this is so, the measured absorption will depend markedly on the flame temperature and the use of a hotter flame should, by increasing the dissociation of the magnesium oxide, lead to increased sensitivity. A small increase can be obtained by using an excess of acetylene in the flame, which presumably increases the dissociation of magnesium oxide by decreasing the free oxygen concentration. With the 7·5-cm burner, an acetylene flow of 1·56 litres per minute (sufficient to render the lower 1 inch of the flame luminous) increased the absorption by about 25 per cent.

The results obtained with the 2·1-cm burner are about six times as sensitive as those reported by Russell, Shelton and Walsh,[2] who used a coal gas flame and a 2-cm Meker burner. This greater sensitivity is, no doubt, caused by a combination of the factors discussed above. In general, the sensitivities reported here are adequate for most magnesium analyses—at least for agricultural materials.

REPRODUCIBILITY—

Provided that the air and acetylene pressures, the photomultiplier voltage and the hollow-cathode current are adequately controlled, duplicate readings agree to better than 1 per cent., as in emission flame photometry.

In order to test the possibility of calibrating the galvanometer directly in magnesium concentration instead of using standard solutions to plot a calibration curve for each set of samples, five solutions containing 0·3, 1·0, 3·0, 6·0 and 10·0 p.p.m. of magnesium were analysed twenty times over a period of 1 week, the 4·8-cm burner being used. During this period, the apparatus was dismantled several times to allow the spectrograph to be used for other purposes. The coefficients of variation of the apparent magnesium concentration were 1·7 per cent. for solutions in the middle of the range, 3·0 per cent. for the solution containing 10 p.p.m. and 6·0 per cent. for the solution containing 0·3 p.p.m. When this experiment was carried out, the power supply for the hollow-cathode lamp was not fully stabilised and the known variations in the current would account for about half the variation in the measured absorption.

I have since found, however, over a more extended period, that the nature of the emission from the hollow-cathode lamp does change, a decrease in intensity of the magnesium emission being accompanied by a sharpening of the magnesium line. The result of this is that now, after several months' use on routine analyses, calibration curves are about 30 per cent. more sensitive than those reported here. My experience has been confined to one lamp only and, of course, it may not be typical.

ACCURACY—

As the number of excited atoms is always a small fraction of the total number of atoms present in the flame, variations in their number, and hence in their emission, caused by the presence of other elements in the flame, will have no effect on the number of unexcited atoms and hence on the measured absorption.[1] Potassium and calcium, both at a concentration of 200 p.p.m., did not affect the absorption of a series of solutions containing from 0·3 to 10·0 p.p.m. of magnesium.

Inaccurate results could, however, arise from two causes. In the first place, other elements could interfere by emitting light sufficiently close to the magnesium wavelength to be passed by the monochromator and sufficiently intense, in comparison with the hollow-cathode lamp, to be detected. The only likely element is sodium, which emits a weak line at 2853 A. Under the experimental conditions described, exactly the same absorption was obtained from 2·0 p.p.m. of magnesium in 0·1 *N* hydrochloric acid as from the same concentration of magnesium in 10 per cent. w/v sodium acetate solution (a commonly used soil extractant containing approximately 17,000 p.p.m. of sodium). Had interference by the sodium emission occurred, it could, of course, have been overcome by the use of a chopper and tuned amplifier as suggested by Walsh.[1] This lack of interference is in marked contrast to the situation that occurs in the determination of magnesium by flame-emission methods, where the degree of interference by sodium is considerable and depends on the resolution

of the monochromator. In the usual Lundegårdh method, in which a medium spectrograph with an entrance slit of 0·04 mm is used, interference by sodium is detectable when the sodium is present at a concentration ten times that of the magnesium, and the analysis of sodium acetate extracts of soil for magnesium is impractiable.

The second way in which inaccurate results could arise is by an alteration in the concentration of magnesium atoms. This would affect the emission and absorption equally and could be caused either by the solution being sufficiently different, in surface tension, viscosity and so on, from the standard solution to affect the atomisation or by the presence in the solution of elements that combine chemically with magnesium in the flame.

The magnitude of the first effect depends on the type of atomiser used. With the Lundegårdh atomiser used in this work, it is found that, in the analysis of *N* ammonium acetate solutions, results are obtained that are about 5 per cent. low when standard solutions in 0·1 *N* hydrochloric acid are used. This same depression occurs in emission flame photometry for potassium, sodium and calcium and can, of course, be readily overcome by using standards that are sufficiently similar in physical characteristics to the samples.

Depression of magnesium emission by sulphate, phosphate and aluminium has been reported by various workers. If, as seems likely, this is caused by formation in the flame of compounds less readily volatilised and dissociated than the magnesium salt used to prepare the standard solutions, then the magnitude of the effect will depend on the flame temperature and also on the fineness of the spray introduced into the flame by the atomiser. In the present instance, no depression in either emission or absorption has been caused by sulphate (up to 0·1 *N* sulphuric acid) or by phosphate (up to a concentration of phosphorus equal to sixty times the magnesium concentration). Aluminium causes a depression both in absorption and emission and must be removed if present in the samples at a concentration comparable with that of the magnesium. This depression is shown by the following results—

Aluminium concentration, p.p.m.	0	2	4	10	20	40
Apparent magnesium concentration, p.p.m. ..	2·50	2·40	2·33	2·18	2·03	1·83

APPLICATION—

Provided that sufficient regard is paid to physical properties, which could influence the atomisation, and to the presence of chemical constituents, which could combine with magnesium in the flame, this method should be applicable to any solution that contains magnesium.

Work in this laboratory is mainly concerned with the determination of magnesium in agricultural materials, and the method has been successfully applied to the analysis of plant-ash solutions, soil extracts, lysimeter and drainage waters, blood sera and milks. The sensitivity of the method is such that soil extracts and water samples can be analysed without the prior concentration necessary for analysis by emission methods For blood serum, the normal range of magnesium concentration can be covered by using a solution obtained by dissolving the ash from 1 ml of serum in 25 ml of 0·1 *N* hydrochloric acid.

CONCLUSIONS

The atomic-absorption method for the determination of magnesium has proved to be rapid, accurate and sufficiently sensitive for most purposes. By its use, magnesium determinations can be performed with the same ease as, and probably with greater reliability than, flame-photometric determinations of sodium, potassium and calcium.

I gratefully acknowledge the assistance of Miss L. Wood and Mr. O. E. Clinton with this work and thank Mr. D. F. Waters for his constant interest and encouragement.

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The Polarographic Determination of Thallium, Iron and Copper in High-purity Cadmium Metal

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A polarographic technique is described by which traces of thallium and iron can be co-determined, after extraction of the trichlorides, in essentially the same base electrolyte. Copper does not interfere at the concentrations under consideration and is separately determined by an extraction procedure with sodium diethyldithiocarbamate. An alternative polarographic procedure is described for the determination of copper, in which an ammonium chloride - ammonium hydroxide base electrolyte is used.

THE methods decribed in this paper were devised to provide a rapid and accurate procedure for the determination of traces of thallium in high-purity commercial cadmium. Pure cadmium might contain, in addition to traces of thallium, comparable traces, *i.e.*, from 0·0002 to 0·0010 per cent., of iron, copper, lead, zinc, tin, nickel and silver, with possibly smaller amounts of antimony, arsenic, tellurium and indium.

Spectrochemical solution methods in current routine use in these laboratories do not permit the accurate determination of concentrations of thallium below about 0·001 per cent. Consideration of the nature and concentrations of the other trace constituents suggested an extraction - concentration approach for thallium. The possibility was therefore indicated of including iron in a scheme for thallium by utilising the solubilities of the trichlorides in diethyl ether. A further advantage would consequently result, since the recommended non-spectrographic method for iron determination is tedious and necessitates a preliminary extraction of the thiocyanate compound with a mixture of pentanol and diethyl ether. Interference by copper might be anticipated in a diethyl ether extraction of the trichlorides of iron and thallium, but this apparently does not occur at the concentrations involved. Preliminary experiments on the removal of interference by copper indicated that an extraction method for its determination was satisfactory.

Since the original investigations by Noyes *et al.* on the relative solubilities of thallic and thallous salts,[1] extensive use has been made of the solubility of thallic chloride in diethyl ether as a preliminary step in the determination of traces of the metal. In consequence, detailed accounts exist of the applications of this method in various fields.[2]

Two publications[3,4] and the Hudson Bay Company's Methods of Analysis describe successful polarographic methods for the determination of thallium; these methods depend on the cathodic reduction of the thallous ion. The fact that the half-wave potential of this reduction remains remarkably constant when different base electrolytes are used has extended its application in defining potential ranges in polarographic analysis. None of the base electrolytes described was found to be a satisfactory medium for the determination of thallium and iron. Considerable promise was shown, however, by polarography in a sodium chloride - ammonium acetate buffered base electrolyte, similar to the type proposed by Semerano and Gagliardi[5] for the determination of greater amounts of lead, zinc and iron. This base electrolyte has frequently been used in these laboratories for the determination of small amounts of lead and iron in tailings from lead and zinc flotation - concentration processes. It is, in our experience, the only base electrolyte that permits satisfactory polarography of iron in concentrations from 0·1 to 40 per cent.

EXPERIMENTAL

POLAROGRAPHIC DETERMINATION OF THALLIUM—

A base electrolyte solution, buffer solution and standard thallium solution were prepared as described on p. 475.

Four 10-ml portions of base electrolyte solution were placed by pipette in 50-ml squat beakers. Standard thallium solution was added to give 25, 50, 75 and 100 μg of thallium,

respectively, in the beakers. These solutions were warmed on a hot-plate until crystallisation of sodium chloride occurred. One millilitre of buffer solution was added to each beaker and the volume of each solution was adjusted to 10·0 ml in a calibrated flask. These solutions, without further treatment, were polarographed on a Tinsley 158 instrument with a static mercury anode and a cathodic drop-rate of 3·0 seconds. The diffusion currents were measured at a half-wave potential of 0·5 volt; the results were as follows—

Thallium added, μg..	25	50	75	100
Diffusion current, μA × 10^2	3·5	8·0	12·5	16·5

A linear relationship can be observed between the thallium content and the diffusion current.

EXTRACTION OF THALLIUM FROM CADMIUM METAL—

A 50-g sample of Specpure cadmium was dissolved in a mixture of nitric and hydrochloric acids and the solution was evaporated to dryness on a hot-plate. Twenty millilitres of hydrochloric acid were added to the residue and the evaporation was repeated. The resulting cadmium chloride was dissolved in water and the solution divided into five equal aliquots. To each of these aliquots was added, respectively, 0, 25, 50, 75 and 100 μg of thallium in the form of a standard solution. These solutions were made 8 *N* with respect to hydrochloric acid, and sufficient bromine water was added, dropwise, to give each a distinct yellow colour. Each of the solutions was then extracted with five 10-ml portions of pure diethyl ether. The extracts were combined and washed with three 20-ml portions of distilled water. The washed extracts were evaporated to dryness in a water bath and 10 ml of base electrolyte solution were added to each. Heating in the water bath was continued until crystallisation of sodium chloride occurred. The solutions were cooled to room temperature and to each was added 1·0 ml of ammonium acetate buffer solution, after which the volume was adjusted to 10·0 ml. These solutions were then polarographed as before, the diffusion currents again being measured at a half-wave potential of 0·5 volt; the results were as follows—

Thallium added, μg	0	25	50	75	100
Diffusion current, μA × 10^2	Nil	4·0	7·5	12·0	16·0

Comparison of these results with those obtained by the polarographic determination of thallium alone indicates that the combined extraction and polarographic method is completely satisfactory.

EXTRACTION OF THALLIUM FROM CADMIUM SOLUTIONS CONTAINING COPPER AND IRON—

Standard solutions were again prepared as in the previous experiments, each containing, in addition to the same fixed amount of thallium, 200 μg each of copper and iron. Spot tests with sodium diethyldithiocarbamate solution, carried out on the dried residue from the evaporation of the ether extract, indicated the complete absence of copper, and tests carried out on the residual solution after extraction of thallium indicated the absence of iron. The presence of iron in the ethereal solution was confirmed by testing with ammonium thiocyanate solution.

From the results of these observations it was apparent that thallium and iron could be quantitatively removed by extraction of their chlorides from 8 *N* hydrochloric acid with diethyl ether, copper, to the maximum concentration expected in high-purity cadmium, being left in the aqueous solution.

EXTRACTION OF THALLIUM FROM HIGH-PURITY COMMERCIAL CADMIUM METAL—

Several samples of high-purity cadmium containing traces of copper, iron, thallium, silver, nickel, lead, zinc, tin, antimony, indium and tellurium, which had previously been spectrographically analysed in these laboratories, were dissolved, extracted and polarographed as previously described. To the solutions of the samples were then added small amounts of thallium corresponding to approximately 50 per cent. of the expected thallium contents. These spiked solutions were treated in the same way, their thallium contents being finally assessed by the polarographic method. The results of four determinations are shown in Table I.

TABLE I

POLAROGRAPHIC DETERMINATION OF THALLIUM IN HIGH-PURITY CADMIUM

The initial thallium content of each sample, determined spectrographically, was $< 0{\cdot}001$ per cent.

Original diffusion current due to thallium, $\mu A \times 10^2$	Amount of thallium added, μg	Final diffusion current due to thallium, $\mu A \times 10^2$	Diffusion current due to added thallium, $\mu A \times 10^2$	Calculated amount of thallium in original sample, %
13·0	50	19·5	6·5	0·00092
7·5	50	14·0	6·5	0·00055
12·0	50	19·0	7·0	0·00086
12·5	50	19·0	6·5	0·00089

From a comparison of the results in Table I with the results for the determinations of thallium alone and in the presence of Specpure cadmium, it is apparent that the method is sufficiently accurate to permit the determination of thallium in a 10-g sample to within $\pm$ 0·0002 per cent.

POLAROGRAPHIC DETERMINATION OF IRON—

The polarography of iron has been extensively examined in these laboratories, and it has been shown that a method enunciated by Semerano and Gagliardi[5] could, in principle, be accurately applied to the determination of the low iron contents of zinc and lead concentrates and tailings. A feature of even the purest obtainable sodium chloride used in this type of base electrolyte is its high blank contribution. Commercial sodium chloride of allegedly high purity has been found often to contain as much as 0·002 per cent. of iron, and, although corrections can be made for this figure in routine analyses involving several milligrams of iron, its presence cannot be tolerated in the microgram range. Extraction of the base electrolyte with diethyl ether has been shown effectively to decrease the blank value to nearly one-tenth of its initial value, but the low inherent acidity of the base electrolyte makes extraction impossible beyond this point.

The method adopted for the purification of the base electrolyte was as follows—

> The base electrolyte solution was prepared only as required, and addition of the gelatin was omitted until after the extraction. Twenty-millilitre aliquots were extracted, after the addition of bromine water, with six successive 10-ml portions of diethyl ether. The extracted solution was exposed to the atmosphere in an open beaker for 30 minutes to permit the evaporation of the small amount of ether that gravitational separation did not remove. The requisite amount of gelatin solution was then added and the solution was stored ready for use. A blank determination of its purity was carried out before each series of determinations.

To 10·0-g samples of Specpure cadmium metal, treated as described for the extraction experiments for thallium, were added 25, 50, 75 and 100 μg, respectively, of iron in the form of a standard solution. The solutions were extracted and polarographed in the same way as for thallium, but with omission of the ammonium acetate buffer solution, the diffusion currents being measured at a half-wave potential of $-0{\cdot}15$ volt; the results were as follows—

Iron added, μg	25	50	75	100
Diffusion current, $\mu A \times 10^2$	1·3	2·5	3·7	5·0

These results confirmed that the sodium chloride base electrolyte provides satisfactory polarography for iron down to microgram amounts.

EXTRACTION OF COPPER—

The generally applied method for the determination of traces of copper is colorimetric, involving complex formation with sodium diethyldithiocarbamate. The yellow colour produced by the complex obeys Beer's law and can be quantitatively measured in aqueous or carbon tetrachloride solutions. Interference has not been experienced when this reaction has been applied to solutions prepared from high-purity cadmium and containing only those elements previously listed, provided that sufficient citric acid has been added before development of the colour. Originally, it was expected that copper would interfere with the thallium

determination by exhibiting a slight chloride solubility in diethyl ether, and, for this reason, its removal by an extraction method was proposed. However, after copper had been shown to have no effect on the accuracy of the thallium determination, the method was retained, as it was rapid and accurate for determining copper. The use of spectrophotometric instruments and standard graphs is replaced by a simple titration that offers almost as high a degree of accuracy. The extraction method for copper can be replaced by the well established polarographic procedure in an ammoniacal base solution after extraction as the diethyldithiocarbamate complex.

Method

Reagents—

All reagents must be of recognised analytical grade.

Citric acid solution, 20 per cent. aqueous.

Sodium diethyldithiocarbamate solution, 0·05 per cent. aqueous—This solution must be freshly prepared.

Carbon tetrachloride.

Ammonium chloride - ammonium hydroxide base electrolyte solution—Mix equal volumes of 4 *M* ammonium chloride and 4 *M* ammonium hydroxide.

Gelatin solution, 1·0 per cent. aqueous.

Hydrochloric acid solution, 8 N.

Bromine water.

Diethyl ether.

Sodium chloride - hydrochloric acid base electrolyte solution—Dissolve 250 g of sodium chloride and 10 ml of a 0·5 per cent. aqueous solution of gelatin in 0·1 *N* hydrochloric acid, and dilute to 1 litre with 0·1 *N* hydrochloric acid.

Ammonium acetate buffer solution—Dissolve 386 g of ammonium acetate and 286 ml of glacial acetic acid in water, and dilute the solution to 1 litre.

Standard copper solution—Dissolve 0·01 g of Specpure copper powder in concentrated nitric acid, add concentrated sulphuric acid and evaporate the solution until fumes are evolved. Dilute the solution to 1 litre.

Standard iron solution—Dissolve 0·01 g of Specpure iron wire in the minimum amount of concentrated hydrochloric acid, and dilute the solution to 1 litre.

Standard thallium solution—Dissolve 0·01 g of Specpure thallium wire in the minimum amount of diluted nitric acid (1 + 1), and dilute the solution to 1 litre.

Extraction procedure for determining copper—

Dissolve a 10-g sample of metal in concentrated nitric acid, with the addition of small amounts of water to moderate the vigorous reaction. Evaporate the solution to incipient dryness on a hot-plate. Add 20 ml of concentrated hydrochloric acid and again evaporate to dryness. Repeat this procedure, the dried salts thus obtained being nitrate-free chlorides. Add 50 ml of water and warm to dissolve the salts. Cool the solution, add 10 ml of 20 per cent. citric acid and transfer to a 100-ml conical separating funnel. Add sodium diethyldithiocarbamate solution, accurately, 0·5 ml at a time, shaking thoroughly after each addition. After each addition, extract the solution with 5-ml portions of carbon tetrachloride until the yellow colour of the copper complex is no longer visible in the organic layer. Record the end of the titration as that point at which the further addition of 0·5 ml of reagent no longer forms a visible yellow colour in the solvent layer. In this way a titre is measured to the nearest 0·5 ml. Since 0·5 ml is approximately equivalent to 10 μg of copper, the method gives a result to the nearest 0·0001 per cent. It is not recommended that the reagent be further diluted for a greater accuracy of titre, but, rather, that if greater accuracy is required, a larger original sample be taken. Standardise the reagent against a solution containing 100 μg of copper and to which citric acid has been added as in the sample analysis.

Polarographic procedure for determining copper—

Transfer the combined carbon tetrachloride extracts to a 100-ml squat beaker and evaporate to dryness in a water bath. To the dried residue add 5 ml of concentrated nitric acid and continue heating on the water bath to destroy the organic matter. Add 5 ml of concentrated hydrochloric acid and heat on a hot-plate until the volume has been reduced to about 2 ml. Add 2 ml of 50 per cent. v/v sulphuric acid and evaporate the solution until

fumes are evolved. Carefully add successive small amounts of nitric acid until all organic matter has been completely destroyed. Evaporate the resulting clear solution almost to dryness. Add 3 ml of water and neutralise the excess of acid by the dropwise addition of ammonia solution, sp.gr. 0·880, use being made of an indicator paper if necessary. Add 2·5 ml of ammonium chloride - ammonium hydroxide base electrolyte solution and 0·5 ml of gelatin solution, and dilute to exactly 10·0 ml. Filter the solution through a Whatman No. 31 filter-paper into a polarographic cell and polarograph as follows—

Range—Apply −0·2 to −0·6 volt against the static mercury anode.
Sensitivity—A full-scale deflection at 4 μA, with counter-current as required and slight damping.
Half-wave potential—Approximately −0·40 volt.

Compare the wave height thus obtained with a graph prepared in the same way from the standard copper solution.

PROCEDURE FOR DETERMINING IRON—

Treat a 10-g sample of metal in the way described under "Extraction Procedure for Determining Copper," as far as the production of a dried chloride residue. Add 50 ml of 8 *N* hydrochloric acid and heat to obtain a clear solution of salts. Add sufficient bromine water, dropwise, to render the solution distinctly yellow, and transfer it to a 100-ml separating funnel. Extract the solution with five 10-ml portions of diethyl ether, combine the extracts and wash them with three 20-ml portions of water. Transfer the washed extract to a 50-ml squat beaker and evaporate to dryness in a water bath. Add 3 ml of concentrated hydrochloric acid to the dried residue and again evaporate to dryness in the water bath. Add 10 ml of sodium chloride - hydrochloric acid base electrolyte solution to the residue and warm to effect dissolution of the salts. Polarograph this solution for iron by applying the necessary counter-current in the range 0·0 to −0·30 volt against the static mercury electrode. Compare the wave height thus obtained with a standard graph prepared in the same way from solutions of Specpure iron wire. If non-routine or very infrequent samples are being analysed, it will probably be better to use the standard-addition comparison technique. By this technique, two solutions are polarographed, the second being identical with the first, but containing a known amount of added reducible ion. The difference between the wave heights thus obtained is proportional to the amount of added ion.

PROCEDURE FOR DETERMINING THALLIUM—

Transfer the polarographic solution, after the iron has been determined, to a 50-ml squat beaker and warm on a hot-plate until crystallisation of sodium chloride occurs. Cool the solution, add 1 ml of ammonium acetate buffer solution and dilute to 10 ml. Polarograph this solution for thallium as follows—

Range—Apply −0·3 to −0·8 volt against the static mercury anode.
Sensitivity—A full-scale deflection at 0·5 μA with maximum counter-current.

Compare the wave height thus obtained with a standard graph prepared in the same way, or use the standard-addition technique.

I thank Mr. Chapman, Consulting Metallurgist for Anglo American Corporation of South Africa Ltd., for permission to publish this paper.

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The Colorimetric Determination of Thallium in Tin - Cadmium Alloys

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A colorimetric method is described for the determination of thallium in tin - cadmium alloys. Microgram amounts over the range 5 to 80 p.p.m. can be determined with an error of ±2 p.p.m.

VARIOUS methods[1 to 7] for determining microgram amounts of thallium were examined during a search for a rapid method of controlling the thallium content of tin - cadmium alloys. None of these methods was found to be satisfactory, mainly because of interference by the large amounts of tin and cadmium present or the lengthy procedures required for the separation of thallium from these elements.

The method described by Pohl,[8] although suitable, has the disadvantage of requiring an ultra-violet spectrophotometer for measuring the absorption of the thallium - sodium diethyldithiocarbamate complex at 315 mμ.

Papers[9,10] describing the use of methyl violet as a reagent for the colorimetric determination of thallium have recently been published. Since the development of the method described, fuller details for the use of this reagent have become available, but experiments indicate that it has no particular advantage over rhodamine B when applied to the determination of thallium in tin - cadmium alloys.

The use of rhodamine B was described by Feigl, Gentil and Goldstein[11] for the detection of thallium. Onishi[12] gave details of a colorimetric method for the determination of thallium, in which the thallic - rhodamine B complex was extracted with benzene. This work has been confirmed by using pure thallous sulphate solutions, but direct application to the determination of thallium in tin - cadmium alloys was not possible, owing to interference from tin and cadmium, which also form complexes with rhodamine B. Separation of thallium was therefore necessary.

Wada and Ishii[13] stated that thallium and gold could be separated from all other elements by extraction with di*iso*propyl ether from 0·1 *N* hydrobromic acid. Preliminary experiments showed that thallium was extracted from 0·1 *N* hydrobromic acid with di*iso*propyl ether and that no co-extraction of tin or cadmium took place. Further experiments showed that the thallic - rhodamine B complex was soluble in di*iso*propyl ether. Tin - cadmium alloy was readily soluble in hydrobromic acid containing 10 per cent. v/v of bromine.

These preliminary experiments indicated that a rapid colorimetric method for the determination of thallium was available and the method was developed for use with tin - cadmium alloys containing 5 to 80 p.p.m. of thallium.

DEVELOPMENT OF THE METHOD

EXTRACTION OF THALLIUM FROM HYDROBROMIC ACID SOLUTION—

The time required for complete extraction of thallic bromide from hydrobromic acid with di*iso*propyl ether was determined by shaking solutions prepared as for the calibration curve (see p. 478) with 25-ml portions of di*iso*propyl ether for increasing periods of time, after which the thallic - rhodamine B complex was formed and the optical density of the extract was measured. Three extracts were measured after being shaken for 1, 3 and 5 minutes, respectively; the optical densities were 0·612, 0·590 and 0·567, from which it can be seen that maximum extraction is attained after 1 minute.

The distribution coefficient was shown to be 1·0 by repeated extraction of a solution of thallic bromide in hydrobromic acid with fresh 25-ml portions of di*iso*propyl ether for 1-minute periods, after which the thallic - rhodamine B complex was formed and the optical density of the extract was measured. The optical densities of the first extracts of two samples were 0·613 and 0·608, and in both, after a second extraction, the optical density was zero.

FORMATION OF THALLIC - RHODAMINE B COMPLEX IN DI*ISO*PROPYL ETHER—

By variation of the shaking time of the di*iso*propyl ether extract - rhodamine B mixture, the time required for formation of the complex was determined. After they had been shaken

for 1, 3 and 5 minutes, respectively, the optical densities of three samples were 0·608, 0·604 and 0·590, from which it can be seen that the complex solution reached maximum optical density after 1 minute.

The decrease in optical density with increased shaking time is considered to be caused by the slow mutual dissolution of the di*iso*propyl ether - hydrochloric acid mixtures, which results in a slight shift of the distribution coefficient as observed at 1 minute.

COLOUR STABILITY OF THE THALLIC - RHODAMINE B COMPLEX—

The stability of the complex in di*iso*propyl ether was determined by measuring the optical density of the coloured solution after known periods of time. After 0, 1 and 15 hours, the optical densities of two solutions were 0·610 and 0·615, 0·610 and 0·613 and 0·605 and 0·609, respectively. These figures show that the complex is stable for at least 1 hour and that the decrease in optical density after 15 hours is only slight.

METHOD

APPARATUS—

The maximum absorption of the thallic - rhodamine B complex occurs at 550 mμ. Any type of spectrophotometer or absorptiometer is suitable; the work described was carried out in 2-cm cells with a Spekker H760 absorptiometer, Kodak No. 5 green filters being used.

REAGENTS—

Hydrobromic acid - bromine mixture—Add 25 ml of bromine to 225 ml of hydrobromic acid, sp.gr. 1·46 to 1·49. Use analytical-reagent grade materials and mix well.

Rhodamine B solution—Dissolve 0·2 g of rhodamine B in 1 litre of *N* hydrochloric acid.

Diisopropyl ether—Analytical-reagent grade.

PROCEDURE—

Into a 100-ml beaker weigh accurately 0·25 g of sample. Add 5 ml of hydrobromic acid - bromine mixture, immediately cover the beaker with a watch-glass to prevent losses by spitting, and heat to approximately 40° C to effect complete dissolution of the alloy. Rinse the watch-glass and beaker walls with 5 ml of distilled water, heat the solution at a temperature just below its boiling-point until excess of bromine has been expelled and then cool to room temperature. Transfer the solution to a 100-ml stoppered separating funnel, graduated at 25 ml, and dilute to 25 ml with distilled water. Add 25 ml of di*iso*propyl ether and shake for exactly 1 minute. Allow the phases to separate, and discard the aqueous layer. Add 20 ml of rhodamine B solution to the ethereal solution and shake for exactly 1 minute. Allow the phases to separate, discard the aqueous layer and transfer the ethereal extract to a dry stoppered cylinder. Note that transfer of the ethereal extract to the dry cylinder prevents the introduction of droplets of the aqueous phase into the absorptiometer cells.

Carry out a reagent blank determination simultaneously with the sample. Measure the optical density of the sample extract in 2-cm cells against the blank solution with a spectrophotometer or absorptiometer.

From the calibration curve, determine the number of micrograms of thallium in the ether extract.

PREPARATION OF CALIBRATION CURVE—

Measure 5 ml of hydrobromic acid - bromine mixture into each of six 100-ml beakers. Add, by pipette, 0·0, 0·2, 0·5, 1·0, 1·5 and 2·0 ml, respectively, of thallous nitrate solution containing 10 μg of thallium per ml to the beakers. Add 5 ml of distilled water to each beaker, heat gently until excess of bromine has been expelled and cool to room temperature. Continue as described under "Procedure" from "Transfer the solution to a 100-ml stoppered separating funnel. . . ."

Measure the optical densities of the ether extracts in 2-cm cells with a spectrophotometer or absorptiometer. Construct a calibration curve of micrograms of thallium against optical density. A linear relationship is obtained with a sensitivity of 0·3 μg of thallium per 0·01 unit of optical density.

RESULTS

Recovery experiments were carried out by adding known amounts of thallium, as thallous nitrate solution, to 0·25-g portions of thallium-free tin - cadmium alloy dissolved

in 5 ml of hydrobromic acid - bromine mixture. The recoveries, which are considered to be satisfactory, were as follows—

Thallium added, μg	5·0	5·0	10·0	10·0	15·0	15·0	
Thallium found, μg	5·2	5·2	10·1	10·3	14·7	14·8	
Recovery, %	104	104	101	103	98	99	

The reproducibility of results was determined by applying the method to a batch of tin - cadmium alloy containing a nominal 40 p.p.m. of thallium. The results were as follows—

Weight of sample, g ..	0·2484	0·2678	0·2527	0·2399	0·2470	0·2504	
Thallium found, p.p.m. ..	43	43	45	44	44	46	(mean, 44)
Mean deviation, p.p.m. ..	−1	−1	+1	0	0	+2	

Conclusion

It is considered that the method is adaptable to the determination of thallium in the presence of a large number of interfering elements by virtue of the selectivity of the extraction procedure.

I thank Standard Telephones and Cables Ltd. for permission to publish this paper.

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Notes

VITAMIN CONTENTS OF ANIMAL FEEDINGSTUFFS

Progress in animal nutrition has led to the widespread use of vitamins of the B complex as supplements to animal rations. The proportion in which any one of these vitamins needs to be

Table I

Milligrams of nutrient per kilogram of feedingstuff*

Feedingstuff	Pro-vitamin A†	Ribo-flavin	Nico-tinic acid	Panto-thenic acid	Pyri-doxin	Choline	Vitamin B_{12}
Wheat	—	1·1	59·4	13·0	4·4	999	—
Barley	—	1·1	70·4	3·5	4·2	1797	—
Oats	—	1·1	8·8	5·7	2·2	1496	—
Yellow maize	0·8	1·1	2·2	3·3	4·6	550	—
Lucerne meal	107	14·1	70·4	26·0	8·8	1896	0·02
Grass meal	137	12·5	55·0	33·9	6·6	1346	—
Dried peas	—	2·9	26·4	13·0	6·4	1298	—
Locust bean	3·2	0·4	17·6	0·7	4·6	51	—
Linseed	—	2·2	41·8	8·4	9·0	1547	—
Ground nut	—	2·2	299·2	31·0	9·9	1797	0·01
Palm kernel cake	—	0·9	11·0	2·2	2·0	229	0·02
Palm kernel meal	—	1·1	11·0	2·2	3·5	319	—
Copra meal	—	1·1	28·6	4·8	3·1	51	—
Undecorticated cottonseed ..	—	1·5	22·0	7·9	3·3	1098	0·02
Decorticated cottonseed ..	—	2·6	26·4	4·0	6·6	2594	0·01
Dried brewers' yeast	—	42·9	378·4	17·8	15·2	5588	0·02

* The figures shown can be converted to milligrams per pound, a form of expression commonly used in Britain, by multiplying by 0·45.

† The amount of vitamin A (in international units per kilogram) approximately equivalent biologically to a pro-vitamin A figure is obtained by multiplying the latter by 1667.

incorporated in a ration depends upon the amount of it naturally present in the ingredients of the ration. Unfortunately, published information on the vitamin contents of common feedingstuffs is very meagre, and we have, therefore, extended existing data by determining the amounts of a number of water-soluble vitamins in a series of common feedingstuffs, and the pro-vitamin A (β-carotene) content of four of them. Comparison of our figures (see Table I) with those collected from the literature and recorded by Kent-Jones and Amos[1] reveals differences that emphasise the need to extend even further the figures for the vitamin contents of feedingstuffs in order more readily to decide the influence of the composition of the ration upon the minimum levels of supplementation for satisfactory nutrition of various types of livestock.

The microbiological assay procedure used for the determination of nicotinic acid, riboflavin, pantothenic acid, pyridoxin and vitamin B_{12} and the chemical method for the determination of choline were those described by Kent-Jones and Amos.[1] Carotene was determined by the method of Booth,[2] which excludes other carotenoids: in most animal feedingstuffs this can be assumed to be β-carotene.

We thank Mr. E. C. Apling and Mrs. J. Whitten for undertaking many of the determinations.

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FUNCTION OF THE SULPHYDRYL GROUP IN THE DETERMINATION OF THIAMINE WITH 6-AMINOTHYMOL

The yellow colour produced when thiamine is treated with 6-aminothymol in alkaline solution has been used recently in a method for determining the vitamin in amounts of the order of 10 to 50 μg.[1,2] The results of an investigation of the reactions involved in this method indicate that the sulphydryl group is the main functional group in the formation of the colour, and that other sulphydryl compounds can be determined in a similar manner.

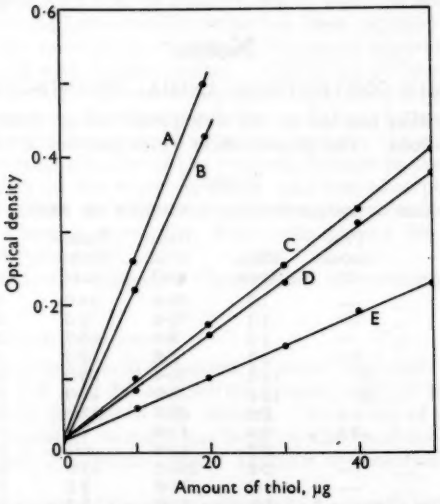


Fig. 1. Reaction of aminothymol with thiols: curve A, thio-*p*-cresol; curve B, thiosalicylic acid; curve C, thiamine; curve D, cysteine; curve E, thioglycollic acid

FUNCTION OF THE SULPHYDRYL GROUP—

It has been reported that certain substances other than thiamine, notably those of a phenolic character, form coloured products with alkaline solutions of aminothymol. Only one of these, cysteine, formed a yellow colour in a similar concentration range to that of thiamine. On the assumption that the colour was attributable to the presence of a sulphydryl group, the test was applied to a number of different thiols. Fig. 1 shows the response of the reagent to these substances under the conditions described for the determination of thiamine.[2] Thio-*p*-cresol formed a turbid solution, and the product was extracted into toluene - butanol mixture (9 + 1) from acid solution before optical-density measurements were made.

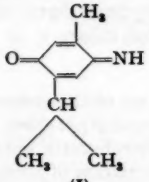
The orange-yellow colours produced with aromatic sulphydryl compounds are very intense and more stable than the greenish yellow colours of the non-aromatic series, but none of the compounds examined gave any measurable colour after treatment with iodine in acid solution.

From these results it was concluded that fission of the thiazole ring of the thiamine molecule occurs under the influence of alkali, and the thiol so formed reacts with aminothymol. According to Williams and Ruehle,[3,4] quaternary thiazolium salts are split at the C–S linkage by an excess of alkali with the formation of a thiol-aldehyde. In order to apply the aminothymol test, a sample of 4-methyl-5-(β-hydroxyethyl)thiazole was prepared by sulphite cleavage of thiamine; neither this nor the pyrimidine portion of the thiamine molecule showed any positive reaction with aminothymol, but, on conversion to its ethiodide, the thiazole portion formed a colour practically identical with that formed by thiamine itself. The reactivity of this compound was also destroyed by treatment with iodine. Downes and Sykes[5] have prepared a disulphide by treating 3-benzyl-2:4-dimethylthiazolium bromide with alkali and iodine.

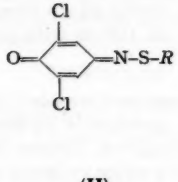
FUNCTION OF AMINOTHYMOL—

For the reaction between aminothymol and a thiol to proceed quantitatively, the presence of free oxygen is essential. This is not evident at low concentrations unless the solutions are de-gassed before mixing, but, at higher levels, vigorous aeration is necessary. Since oxidised thiols do not respond to the test, it is clear that aminothymol requires at least partial oxidation before the reaction can take place. This is borne out by the observation that preliminary aeration of alkaline solutions of aminothymol greatly accelerates colour development when a thiol is added.

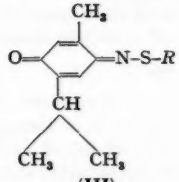
The most probable oxidation product of 6-aminothymol in the early stages of the reaction is thymoquinoneimine (**I**). Undoubtedly this reaction does not reach completion, ammonia being slowly liberated, and, after standing for about 1 hour, the solution no longer reacts with thiamine. McAllister[6] has assigned the structure **II** to the yellow product obtained in alkaline solution from 2:6-dichloroquinonechloroimide and mercaptoglyoxalines, and it is suggested that thymoquinoneimine reacts similarly under mildly oxidising conditions to form the compound **III**.



(I)



(II)



(III)

Although McAllister formulates the structure **II** on the basis of Gibbs's reaction for phenols,[7] certain experimental observations suggest a reaction that involves the imino group rather than the ketonic group. For example, it has been found that replacement of the imino hydrogen by an alkyl group destroys completely the reactivity of the quinoneimine towards thiols. Further, although 4-amino-2:6-xylenol is as sensitive to thiols as aminothymol, 4-amino-3:5-xylenol is devoid of activity. Saunders and Watson[8] have pointed out that, owing to steric effects, the reactivity of quinones is inhibited by the presence of *o*-methyl groups.

A more detailed investigation of the yellow products of the reaction between aminothymol and sulphydryl compounds is made difficult by their extreme instability. The action of mineral acids, warming or even standing in neutral solution for a few hours causes rapid decomposition, which leads to the formation of disulphides and coloured phenolic oxidation products. The data presented are in agreement with the N-mercaptoquinoneimine structure proposed by McAllister, and indicate that the reaction may be applicable generally as a method for the photometric determination of free or derived thiols.

I thank the Directors of Novadel Ltd. for permission to publish this Note.

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K. J. Hayden
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VOLUMETRIC DETERMINATION OF SOME ORGANOCHLOROSILANES WITH AMMONIUM THIOCYANATE

The observation by Gingold and Rochow[1] that ammonium thiocyanate will undergo double decomposition with dimethyldichlorosilane to precipitate ammonium chloride prompted the direct determination of some organochlorosilanes in organic solvents with ammonium thiocyanate.

Details of the procedure and the results of the experiments carried out to establish a new simple technique for determining the concentration of some organochlorosilane solutions directly by means of precipitation with a solution of ammonium thiocyanate in acetone in presence of ferric chloride solution as indicator are given.

Although it is fairly simple to determine these chlorosilanes by titration of the acidity, or chloride ion, after hydrolysis with water, the proposed method has the advantage that it is carried out entirely in organic solvents and no precautions are needed against possible loss of hydrochloric acid, which might occur if adequate precautions were not taken in the aqueous hydrolysis method.

Method

Reagents—

Ammonium thiocyanate solution, 0·3 M in acetone—Dissolve 22·8 g of ammonium thiocyanate (dried in a vacuum-desiccator after recrystallisation from methanol) in 100 ml of warm anhydrous acetone, and dilute with more anhydrous acetone to 1 litre. Standardise the solution against a 0·05 *N* aqueous solution of silver nitrate.

Ferric chloride solution, 1 per cent. in diethyl ether—Dissolve 1 g of anhydrous ferric chloride in 10 ml of absolute ethanol, and dilute to 100 ml with anhydrous diethyl ether.

Procedure—

With use of a pipette calibrated by means of mercury, place exactly 1 ml of the chlorosilane sample solution in a 100-ml conical flask, and add 10 to 30 ml of anhydrous diethyl ether. Add 2 or 3 drops of 0·3 *M* ammonium thiocyanate in acetone from a 5-ml microburette graduated in 0·01 ml; the solution should now assume a white turbidity owing to the formation of ammonium chloride. Shake the solution and add 1 drop of 1 per cent. ferric chloride solution in diethyl ether. Continue the titration to the appearance of a persistent red colour owing to the formation of ferric thiocyanate.

It is necessary to shake the flask vigorously during the titration and to keep the contents below 5° C by using iced water. Practice is, at first, required for accurate detection of the end-point, but reproducible results should soon be achieved.

Calculate the concentration of chlorosilane in the sample solution (*C*) from the following equation—

$$C = \frac{A \times V \times 354{\cdot}57}{B} \text{ per cent. w/v,}$$

where A = the molar concentration of the ammonium thiocyanate solution,
V = the volume, in ml, of ammonium thiocyanate solution used (mean of at least five titrations), and
B = the chlorine content, in per cent. w/w, of the chlorosilane.

Results and discussion

The proposed procedure was applied to solutions of twelve different chlorosilanes. The results, together with the calculated values, are shown in Table I. Methylchlorosilane and phenylchlorosilane were supplied by the Shin-etsu Chemical Industrial Company in purified grades. Ethylchlorosilane[2] and *iso*propylchlorosilane[2] were prepared by Grignard synthesis and then purified by careful single distillation. The sample solutions were prepared by dissolving the weighed chlorosilane in either anhydrous benzene or ethyl acetate.

The results in Table I show that the proposed method is useful and convenient for rapidly determining chlorosilanes in organic solvents, especially when the solvent mixture is of unknown composition so that the usual techniques, *e.g.*, refractometric or gravimetric, cannot be applied effectively.

Throughout the preliminary investigations it was found that addition of a large amount of dry diethyl ether to chlorosilane samples was most effective for the production of a suitably sharp end-point; dilution with diethyl ether, especially when methylchlorosilane or ethylchorosilane was used, made the precipitation of ammonium chloride so complete and the colour owing to the formation of ferric thiocyanate so distinct that 1 drop of ammonium thiocyanate solution in excess was sufficient to make the end-point easily recognisable.

I thank the Shin-etsu Chemical Industrial Company for the supply of some pure chlorosilanes.

Table I

Determination of some chlorosilanes in benzene and in ethyl acetate

Chlorosilane	Solvent	Concentration found, % w/v	Concentration calculated, % w/v
Methyltrichlorosilane*	Benzene	6·70	6·72
	Ethyl acetate	11·26	11·26
Dimethyldichlorosilane*	Benzene	5·08	5·10
	Ethyl acetate	9·90	9·91
Ethyltrichlorosilane	Benzene	17·32	17·37
	Ethyl acetate	4·45	4·52
Diethyldichlorosilane	Benzene	61·70	61·74
	Ethyl acetate	30·37	30·42
*iso*Propyltrichlorosilane	Benzene	20·20	20·32
	Ethyl acetate	12·24	12·37
Phenyltrichlorosilane	Benzene	70·88	71·01
	Ethyl acetate	21·11	21·30

* Some results obtained by the author with these chlorosilanes will soon be published in *J. Chem. Soc. Japan* (*Ind. Chem. Sect.*).

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INCREASING THE PRECISION OF FREEZING-POINT DETERMINATIONS

The freezing or crystallising-point of a material is often used as a criterion of its purity or composition. Determination of the freezing-point by conventional methods, however, is hampered by the tendency of many substances to supercool. To overcome this difficulty, either elaborate arrangements are used to add external seed or else cooling curves are drawn and extrapolated to what is hoped is the true freezing-point of the material. Supercooling can be almost completely avoided in the majority of determinations by a simple addition to the usual apparatus specified in Appendix B of British Standard 1998:1953.

The use of a cooled wire to induce crystallisation is fairly well known.[1] This can be adapted to a form suitable for freezing-point determination by attaching to the wire a small brass cup containing a cooling agent. The wire is passed through the cork of the tube containing the sample

so that the cup is just above the cork. The end of the wire dips 5 to 10 mm into the liquid and is positioned to make contact with the stirrer at each oscillation. During the determination, the brass cup is kept full of a substance, such as ice, solid carbon dioxide or liquid air, which serves as a heat sink some 50° C or so below the freezing-point of the sample. As the sample approaches its freezing-point, the intense cooling at the point of entry of the wire into the liquid causes crystallisation in a small volume of the liquid. The crystals so formed are detached by the stirrer and dispersed throughout the bulk of the sample, thereby ensuring adequate seed at the critical point. Supercooling is usually undetectable, and, if temperature readings are made at suitable intervals of time, the freezing-point is sufficiently marked to obviate the preparation of cooling curves.

The rate of heat abstraction by the device can be varied within wide limits by changing the diameter of the wire and the temperature of the heat sink. The method is thus adaptable in principle to the majority of freezing-point determinations, in particular those that have to be conducted below room temperature.

As an example, the following conditions gave good results with mixtures of ethylene glycol and water, which readily supercool in the absence of seed. A piece of 18 s.w.g. (0·048-inch diameter) copper wire was used, attached to a cup 15 mm in diameter and 20 mm tall, which was filled with small pieces of solid carbon dioxide. The outer bath temperature was maintained between 8° and 14° C below that of the sample in order to achieve a uniform rate of temperature decrease of approximately 0·2° C per minute. The sample was pre-cooled on solid carbon dioxide to obtain an approximate freezing-point value, after which its temperature was allowed to increase by 1° or 2° C before insertion in the apparatus. The temperature was read every 30 seconds, and the first temperature to be followed by four more readings that differed by less than 0·05° C was taken as being the true freezing-point. Duplicate determinations usually agreed to within 0·05° C.

Acknowledgment is made to the Engineer-in-Chief of the Post Office and to the Controller of H.M. Stationery Office for permission to publish this Note. I thank Mr. R. A. Fraser of Benzole Distillers Ltd. for valuable suggestions.

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Book Reviews

Organische Fällungsmittel in der Quantitativen Analyse. By Dr. Wilhelm Prodinger. Fourth Edition. Pp. xvi + 246. Stuttgart: Ferdinand Enke Verlag. 1957. Price (paper) DM.33; (cloth boards) DM.35.60.

Prodinger's "Organic Precipitants in Quantitative Analysis" has won its place on the analyst's bookshelf already, but the author has again been at great pains to summarise all the important advances in this field since the publication of the successful third edition. The coverage is best indicated for new readers by the following list, in which figures in brackets give the number of pages of text devoted to each topic: dipicrylamine (5), sodium tetraphenylborate (10), picrolonic acid (8), anthranilic acid (10), quinaldinic acid (11), cupferron (17), neocupferron (1), N-benzoylphenylhydroxylamine (1), dimethylglyoxime (3), benzoinoxime (6), salicylaldoxime (6), mercaptobenzthiazole (7), oxine (22), α-nitroso-β-naphthol (6), pyrogallol (1), thionalide (16), sulphosalicylic acid (8), guadinium carbonate (3), tannin (14), arsonic acids (10), mandelic acids (2), *m*-cresoxyacetic acid (3), *p*-amino-acetophenone (1), "naphthin" (1(or 4)-azaphenanthrene) (4), ethylenediamine (4), propylenediamine (2), pyridine (10), tolidine and benzidine (2), thiourea (6), tetraphenyl- and triphenylmethyl-arsonium salts (6), nitron (4) and triphenyltin chloride (2).

Certain reagents recently advocated, *viz.*, violuric acid, bismuthiol II, phenarsazinic acid and 1-nitroso-2-hydroxy-3-naphthoic acid have not been included, because nothing apart from the original papers has been written about them, and as yet the author has had no personal experience of their potentialities or limitations. This may be the reason for omitting dithiol, phenylselenic acid and some reagents developed by Russian chemists.

This book follows the pattern of its predecessors in giving detailed descriptions of particular separations and quantitative determinations. It is thoroughly practical in outlook and there are repeated indications of the possibility and reasons for errors in certain of the published procedures. The author comments on discrepancies between the optimum drying temperatures for precipitates reported by Duval from studies with his thermobalance and those recommended by other workers who used conventional drying ovens or furnaces of different designs.

This book is well printed, strongly bound and remarkably free from misprints and errors, although the formulae for the complexes of scandium and thorium with 8-hydroxyquinoline (pp. 122 and 123) should have been $Sc(Ox)_3.HOx$ and $Th(Ox)_4.HOx$. No reference is made to Hollingshead's four-volume monograph on "Oxine," and the author has little space to spare for the reagents nioxime, heptoxime or niccolox—and none for furil-dioxime. Although it has long been demonstrated that the correct formula for the complex of thiourea and lead nitrate is $Pb(NO_3)_2.6CS(NH_2)_2$ (Haworth and Mann, *J. Chem. Soc.*, 1943, 661), Mahr's erroneous and improbable formula $2Pb(NO_3)_2.11CS(NH_2)_2$ has not been corrected in this edition.

H. IRVING

PURITY CONTROL BY THERMAL ANALYSIS. Proceedings of the International Symposium on Purity Control by Thermal Analysis, Amsterdam, 1957. Sponsored by the I.U.P.A.C. and organised by the Committee on Physico-Chemical Data and Standards. Edited by W. M. SMIT. Pp. xii + 182. Amsterdam: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd.; New York: D. Van Nostrand Co. Inc. 1957. Price 24s.; $4.85.

Chemical compounds of high purity are required not only for research purposes and as standards for the calibration of instruments, but certain of them are used industrially, for example, titanium tetrachloride and dimethyl terephthalate. Hence, methods of determining purity of substances are of great importance and the development of new methods becomes necessary as standards of purity are raised. During the past 15 years or so, much effort has been directed to the use of thermal properties for measuring the purity of substances. The present state of knowledge of this subject was reviewed at an International Symposium in Amsterdam in 1957 organised by the Committee on Physico-Chemical Data and Standards of I.U.P.A.C. This volume contains the proceedings of the Symposium: sixteen papers, the text of the Chairman's opening address and a summary of the discussion. The papers have previously been published as Volume 17, No. 1, of *Analytica Chimica Acta*.

The papers deal with the theory, applications and techniques of the thermometric method for obtaining melting and freezing curves and of the calorimetric method for measuring temperature - heat-content curves. The analyst will be particularly interested in the papers dealing with general methods, for example, the comparison between thermometric and calorimetric techniques, a critical survey of the calorimetric method and the theory and practice of thermometric methods. Precise measurements in this field require a large number of observations and it is desirable to use automatic control as far as possible. Three papers describe apparatus for the automatic or partly automatic control and recording of thermal analyses. Several of the papers consider the interpretation of experimental results to obtain a figure for the purity of the sample. Three papers are concerned with special applications of the methods.

The discussions at the Symposium are summarised in the last eight pages of the book. It is probable that the melting (or freezing) curve method for purity determination will be much more widely used in the future and it is of interest to note that the Symposium recommended that the reliability of this method be tested by the comparison of the results on standard samples by several interested laboratories.

The volume is well produced and the price is very reasonable. J. F. MARTIN

FLAME PHOTOMETRY. By F. BURRIEL-MARTÍ and J. RAMÍREZ-MUÑOZ. Pp. xii + 531. Amsterdam: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd.; New York: D. Van Nostrand Co. Inc. 1957. Price 65s.

The authors have previously published in Spanish a review of flame-photometry methods and the very great interest shown in that work led them to write the present book designed for English-speaking workers. They set themselves the task of producing a manual that will serve both as a textbook for those who have never used the technique and as a work of reference for those already using flame photometry.

One of the great difficulties in writing a book on this subject is the tremendous variety of instruments available and the almost impossible task of correlating data obtained from them.

In addition, there are in existence large numbers of laboratory-built flame photometers. The authors of the book have coped with this difficulty very well in that they have not gone into too much detail on those problems, particularly interference problems, which depend so largely on instrumental details. They have also pointed out carefully that the analytical procedures that can be undertaken must depend on the type and versatility of the available equipment.

The subject has been treated very fully, in places almost too fully. For instance, although anyone who is particularly interested in instrumentation will delight in the ten pages devoted to detailed comparison of all the known commercial instruments and many of the laboratory instruments that have been described in the literature, many readers may consider the section too detailed.

The book is divided into 6 major divisions plus an appendix, 25 chapters and 117 sections, with further headed sub-divisions within many of the sections and this results sometimes in a somewhat discontinuous text that may not be easily readable to someone new to the subject. To counteract this there is an extremely good subject index, which makes the finding of any particular piece of information quite easy. Although there has been no attempt to provide a complete bibliography, this having been made unnecessary by previous publications by another author, 909 references are given.

The translation by W. C. Darwell is very good indeed; there are few passages that reveal that the book was written originally in Spanish. On its own merit the book is extremely useful, but, in addition, it is the first work on this subject in English, and so it cannot fail to be a most important addition to the library of any modern laboratory. L. BREALEY

A MANUAL OF PAPER CHROMATOGRAPHY AND PAPER ELECTROPHORESIS. By RICHARD J. BLOCK, EMMETT L. DURRUM and GUNTER ZWEIG. Second Edition. Pp. xii + 710. New York and London: Academic Press Inc. 1958. Price $12.80; 91s. 6d.

So rapid have been the advances in this branch of analytical chemistry that the first edition of this book, published in 1955, has had to be largely re-written. Part I, comprising two-thirds of the whole, is concerned with paper chromatography and is the work of R. J. Block and G. Zweig; the remainder, written by E. L. Durrum, is devoted to paper electrophoresis.

In the introduction the reader is reminded that R. Consden, A. H. Gordon and A. J. P. Martin (*Biochem. J.*, 1944, **38**, 224) were the first to use paper as the inert support in place of the silica gel originally proposed by A. J. P. Martin and R. L. M. Synge (*Biochem. J.*, 1941, **35**, 1358). Following on this pioneer work, numerous investigators have employed the principle of paper partition chromatography to separations of all kinds of closely related substances with striking success, and the purpose of this treatise is to present some of the results, so that the reader may have a knowledge of past studies sufficient to guide him in planning procedures to deal with his own requirements in this field, without recourse to an extensive search of the literature.

After a chapter devoted to an exposition of theoretical principles, there is a useful illustrated account, extending to more than 60 pages, of the methods of paper chromatography. This section is the foundation of the discourse and has been most carefully written. The next chapter, comprising an account of the various methods of quantitative assessment, makes interesting reading and, although naturally including detailed explanations of the senior author's Total Colour Density and Maximum Colour Density methods, the Elution principle, as well as the Area of Spot proposal of R. B. Fisher *et al.* (*Nature*, 1948, **161**, 764), are equally well described. From this point on, for 333 pages, the work is devoted to detailed descriptions of paper-chromatographic methods as applied to various classes of compounds, the titles of the ten chapters being: Amino Acids, Amines and Proteins; Carbohydrates; Aliphatic Acids; Steroids, Bile Acids and Cardiac Glycosides; Purines, Pyrimidines and Related Substances; Phenols, Aromatic Acids and Indole Compounds; Naturally Occurring Pigments; Miscellaneous Organic Substances; Antibiotics and Vitamins; Inorganic Separations. There are some 67 Tables of R_F values, and the work is documented throughout. Part I of the book concludes with a list of approximately 1800 references, a few of these belonging to the year 1957. At the end of the volume there is also an index of about 1180 substances showing the page where the R_F value is quoted.

As stated in the Preface, the authors have not attempted to discuss all the published work on paper chromatography, their object having been to write a practical manual in which tried and proved procedures are summarised. It would appear that most of the substances to which paper chromatography has been applied are mentioned in the text, although, necessarily, in many instances it has only been possible to quote the more important references.

After a chapter on theoretical considerations, the author of Part II skilfully introduces the practical aspects of paper electrophoresis by illustrated descriptions of his own early arrangements of apparatus, and hence readers without previous acquaintance with the technique are able to follow its later developments with ease. An Appendix giving the formulae of the commonly used electrolytes, with notes on them, is particularly useful. There are then 88 pages of bibliography comprising over 1700 references on paper electrophoresis and closely allied topics, and the value of these is enhanced by the appended subject classification. The index at the end of the volume covers Parts I and II.

This book bears the stamp of authority and is certain to stimulate further research in the development of a most valuable, and essentially simple, analytical technique.

NOEL L. ALLPORT

DIE METHODEN DER MIKROMASSANALYSE. By Professor Dr. JOSEF MIKA. Second Edition. Pp. xvi + 375. Stuttgart: Ferdinand Enke Verlag. 1958. Price (paper) DM 63; (cloth boards) DM 66.

The first edition of Mika's textbook was published in 1939, and many developments in volumetric analysis have taken place in the interval. Mika, in this new edition, has re-cast the original work and widened its scope considerably.

It must be emphasised, however, that this publication is not just a handbook that describes all the known methods of micro-titration. On the contrary, it is really a guide to the whole subject and is likely to be of great help to analysts of all kinds who have to carry out volumetric titrations on the macro, micro or ultra-micro scale.

The book is divided into two parts, one of which is general and the other special. In the general part, Mika deals with the purpose of micro-volumetric analysis, the purity of chemicals that are used and methods of end-point detection, including colorimetric, photometric, potentiometric, amperometric, conductometric and high-frequency procedures. He pays particular attention to the various methods available for the delivery of known weights or volumes of volumetric solutions, whether by conventional burette, weight burette, flask burette or capillary or coulometrically.

The preparation of volumetric solutions is described, and observations are made on the most desirable working concentrations, as well as on the subdivisions of solutions.

In the special part, volumetric methods of neutralisation analysis in aqueous and non-aqueous media, oxidation and reduction procedures, titrations involving complex formation and methods involving precipitation are dealt with most thoroughly; examples are given of the applications of the tests.

This book is really first class. Naturally, the bias in this type of book is towards the inorganic side. Nevertheless, it is the kind of publication that must appeal to all analysts who are interested in principle rather than scale and who wish to place the volumetric work of their particular laboratories on a rather firmer basis.

J. HASLAM

TECHNIQUE OF ORGANIC CHEMISTRY. Edited by ARNOLD WEISSBERGER. Volume X: FUNDAMENTALS OF CHROMATOGRAPHY. By HAROLD GOMES CASSIDY. Pp. xviii + 447. New York and London: Interscience Publishers Inc. 1957. Price $9.75; 78s.

This book represents one of the first attempts at a fundamental treatment of all the chromatographic techniques. It covers the following types of chromatogram: liquid - solid (adsorption chromatography), liquid - liquid (miscalled "partition" chromatography), both on columns and paper, and gas - liquid. Curiously enough, no treatment is given of the gas - solid chromatogram.

The subjects dealt with in the fifteen sections are: Introduction; The nature of chromatography; The molecular interactions on which chromatographic separation rest; General theory; Gas - liquid partition chromatography; Column partition chromatography; Paper partition chromatography; Adsorption chromatography; Ion exchange; Electron-exchange polymers; Foam and emulsion fractionation; On recognising and evaluating zones; On the relation of R or R_F to molecular structure; On choosing mobile and stationary phases; On using chromatography; and, finally, an Appendix that gives lists of manufacturers supplying apparatus. The Subject and Cumulative Indexes are adequate.

In a book of this size no subject can be treated comprehensively. Nevertheless, all the basic information for each technique is present. Where information from published papers is dealt with, the relevant sections of original works are quoted in full, so that no author can complain of being misrepresented.

British workers in the field might quibble over Dr. Cassidy's arrangement and classification scheme, but, on the whole, the book does deal fairly adequately with the fundamentals of the chromatographic technique. Wisely, no attempt has been made to cover all the published work on the subject, since a list of the references alone would fill a book of similar size.

Dr. Cassidy's book is a good addition to the literature on the subject and can be recommended to those facing separation problems not already solved, and to those interested in the basic phenomena involved in chromatographic techniques. A. T. JAMES

Publications Received

SOUTH AFRICAN JOURNAL OF AGRICULTURAL SCIENCE. Edited by V. E. DE KOCK. Volume 1, No. 1, March, 1958. Pp. 108. Pretoria: Department of Agriculture. Single copies 7s. 6d.

VIJNANA PARISHAD ANUSANDHAN PATRIKA (THE RESEARCH JOURNAL OF THE HINDI SCIENCE ACADEMY). Chief Editor: Dr. SATYA PRAKASH, D.Sc. Volume 1, No. 1. Pp. 78. Allahabad, India: Vijnana Parishad. Annual Subscription: Rs. 8.00; 12s.; Single copies Rs. 2.00; 3s.

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Sixième Série. Pp. iv + 171. Paris: Masson et Cie. 1958. Price 2600 fr.

PRACTICAL MICROSCOPY. By L. C. MARTIN, D.Sc., A.R.C.S., D.I.C., and B. K. JOHNSON, D.I.C. Third Edition. Pp. xii + 138. London and Glasgow: Blackie & Son Ltd. 1958. Price 12s. 6d.

ADVANCES IN PETROLEUM CHEMISTRY AND REFINING. Edited by KENNETH A. KOBE and JOHN J. MCKETTA, jun. Volume I. Pp. xvi + 641. New York and London: Interscience Publishers Inc. 1958. Price $13.50; 103s.

THE METABOLISM OF SULPHUR COMPOUNDS. By LESLIE YOUNG, D.Sc., Ph.D., F.R.I.C., and GEORGE A. MAW, Ph.D., F.R.I.C. Pp. 180. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1958. Price 16s.

BIG MOLECULES. By SIR HARRY MELVILLE, K.C.B., Ph.D., D.Sc., F.R.S. Pp. 180. London: G. Bell & Sons Ltd. 1958. Price 15s.

Based on the Royal Institution Christmas Lectures.

RECOMMENDED METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS. Prepared by a Joint Committee of the Association of British Chemical Manufacturers and The Society for Analytical Chemistry. Pp. xii + 124. Cambridge: W. Heffer & Sons Ltd., *for* The Society for Analytical Chemistry. 1958. Price 42s.

BIBLIOGRAPHY OF FOOD. A SELECT INTERNATIONAL BIBLIOGRAPHY OF NUTRITION, FOOD AND BEVERAGE TECHNOLOGY AND DISTRIBUTION 1936–56. By E. ALAN BAKER, A.L.A., and D. J. FOSKETT, M.A., F.L.A. Pp. xii + 331. London: Butterworths Scientific Publications; New York: Academic Press Inc. Price 63s.; $11.00.

MODERN ELECTROANALYTICAL METHODS. Edited by G. CHARLOT. Pp. xii + 186. Amsterdam: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd.; New York and Toronto: D. Van Nostrand Co Inc. 1958. Price 24s.; $4.85; Dfl. 12.50.

Reprint of January/February issue of Analytica Chimica Acta, **1958, 18,** *pp.* **1–182,** *with Introduction and Index.*

THE ANALYSIS OF RUBBER AND RUBBER-LIKE POLYMERS. By WILLIAM C. WAKE, M.Sc., Ph.D., F.R.I.C., F.I.R.I. Pp. x + 237. London: Maclaren & Sons Ltd. 1958. Price 50s.; $8.00.

MANUAL OF ANALYTICAL METHODS RECOMMENDED FOR SAMPLING AND ANALYSIS OF ATMOSPHERIC CONTAMINANTS. By the Committee on Recommended Analytical Methods, American Conference of Government Industrial Hygienists. Loose leaf, viii + 50 pages (11 Methods). Cincinnati, Ohio: American Conference of Government Industrial Hygienists. 1958. Price $5.00.

The price includes also the next nine methods that receive Committee approval. Orders or enquiries should be addressed to The Secretary-Treasurer, A.C.G.I.H., 1014 Broadway, Cincinnati 2, Ohio, U.S.A.

AN INTRODUCTION TO THE CHEMISTRY OF FATS AND FATTY ACIDS. By F. D. GUNSTONE, Ph.D., A.R.I.C. Pp. x + 161. London: Chapman & Hall Ltd. 1958. Price 32s.